2010-01317F

国家海洋局第一海洋研究所
海洋环境测试中心
Centre of Marine Environmental Measurements, FIO, SOA

Final report of the Shipboard Trials of the *BSKY*^{TM} Ballast Water Management System

Number: FIO [2011] C0402

Entrust Entity: Wuxi Brightsky Electronics Co., Ltd

Samples: Water quality, Organisms (>10 μm), Microbes

Inspection Institution: First Institute of Oceanography, SOA

Approval: _____

Issue date: Feb 22, 2011

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Centre of Marine Environmental Measurements, FIO, SOA

Final report of the Shipboard Trials of the *BSKY™ BWMS*

Number: FIO [2011] C0402

Entrust Entity	**Name:** Wuxi Brightsky Electronics Co., Ltd	**Contact:** Linxing Wang
	Address: Luoshe, Wuxi, Jiangsu province	**Tel:** +86 0510 83306200
	Entrust date: July, 2010 - January, 2011	**Testing date:** July, 2010 – February, 2011
Sample and item being measured	**Name:** temperature,salinity, NTU, pH, DO, TSS, POC, DOC, organisms(10 μm ~50 μm), organisms(≥50μm), microbes	**Number:** 240, 60 for water quality; 60 for organisms(10 μm ~50 μm); 60 for organisms (≥50 μm); 60 for microbes.
	Numbering and label: SST…series number outside the bottles or Petri dish membrane	**Note** : "a" was added at the end of number for organisms(≥50 μm) samples, such as SST1 -1B1/a. "b" was added at the end of number for organisms(10 μm~50 μm) samples, such as SST1-1B1/b. "c" was added at the end of number for microbes samples, such as SST1-1B1/c. "d" was added at the end of number for water quality samples, such as SST1-1B1/d.
	Received/sampled by: Ping Liu **Sampling date** : July, 2010 - January, 2011	
	Number of submitting list:	

	Program	Parameter	Standard	Method	Device/Type	Testing person
Test	water quality	T, S, pH, DO, NTU, TSS, POC, DOC	Specification for oceanographic survey specification for marine monitoring	pH: pH-metric method, DO:Winkler method, NTU:spectrophoto-metric method, TSS:weight method, POC, DOC: high temperature combustion method	TOC-V_{CPH} POC element analyzer /ELIII, 722S Spectro photo-meter	
	organisms	10 μm~50 μm; ≥50 μm	Specification for oceanographic survey	count with microscope	Nikon-TS100 invert micro Scope,LeicaL2 microscope	
	microbe	Bacteria *Vibrio cholerae*; *E.coli* Intestinal enterococci	Specification for marine monitoring	Plate method Membrane filter method		

result	Appendix 1:Results for chemical parameters of the Shipboard Trials of *BSKY™ BWMS* Appendix 2:Results for organisms (≥50 μm) of the Shipboard Trials of *BSKY™ BWMS* Appendix 3:Results for organisms (10 μm~50 μm) of the Shipboard Trials of *BSKY™ BWMS* Appendix 4:Results for microbes of the Shipboard Trials of *BSKY™ BWMS* Appendix 5:Results for photosynthetic activity of plankton of the Shipboard Trials of *BSKY™ BWMS*

Compiled by		Checked by		Approved by	
Date of compiling	2011.2.22	Date of checking	2011.2.22	Date of Approval	2011.2.22

CONCLUSIONS

The Shipboard Trials of *BSKY*^{TM} *BWMS* manufactured by Wuxi Brightsky Electronics Co., Ltd. was conducted on Huachang 8 Liquefied petroleum Gas Carrier for 4 trials though the summer and winter, which complied with the time requirements of G8. According to the testing results and the reference of G8 and D-2 standard (Table 5.1), the conclusion was made as follows:

1) The organism densities for different size fraction of the 4 Shipboard Trials were various from each other: for the large size fraction (≥50 μm), the density fluctuated between 1.69×10^2 ind/m^3 and 5.89×10^2 ind/m^3, while the value for small size fraction (10 μm ~50 μm) was from 1.19×10^2 cells/ml to 9.32×10^3 cells/ml. All the densities were more than 10 times of the greatest number defined by D-2 standard, as a result, all of the 4 trials were valid.
2) Viable organisms (≥50 μm) were only observed in one sample of the second trial for the effluent water of treated tank during the 4 trials, but the living activity was very weak, it may be die within several hours, no survivals were observed for the other three trials. All the results met the D-2 standard and G8 well.
3) For the viable plankton (10 μm ~50 μm), two samples of the second trials were proved positive for that, the density was 0.04 ind/ml on average, none survivals were observed for the other three trials, the average density for the 4 trials was only 0.01 ind/ml, which met the D-2 standard and G8 requirement.
4) The number of heterotrophic bacteria for each trial was 2.89 CFU/100ml, 12.00 CFU/100ml, 6.22 CFU/100ml and 0 CFU/100ml on average, respectively. There were none viable *Vibrio cholerae* observed after an incubation of sample bottles to the treated water from the 4 trials. The number of Intestinal enterococci and *Escherichia coli* colonies were all less than 1 CFU/100ml after treated which met the D-2 standard and G8 requirements.

All in all, according to the results of the Shipboard Trials of the *BSKY*^{TM} *BWMS*, although several samples of shipboard trial had a slightly higher density of viable organisms than that in the land-based test, the removal effect to different size fraction of organisms still met the D-2 standard and G8 well, and the efficiency of treatment was over 99.9%.

Compiled by		Checked by		Approved by	
Date of compiling	2011.2.22	Date of checking	2011.2.22	Date of Approval	2011.2.22

Final Report of The Shipboard Trials of The *BSKY*^{TM} Ballast Water Management System

Inspection Institution: The First Institute of Oceanography, SOA

Supervisor: China Classification Society

Manufacturer: Wuxi Brightsky Electronics Co., Ltd

Ship for trials: LPG Carrier, Huachang 8

Feb 22, 2011

Contents

1 Introduction

Ships transport 5-10 billion tons of ballast water annually all over the world (Endresen et al. 2004). The ballast water is loaded with particulate sediment and an enormous variety of (living) organisms, which ranges from juvenile stages, larvae and eggs of fish and larger zooplankton (Williams et al. 1988; Carlton & Geller 1993) to macroalgae, phytoplankton (Hallegraeff et al. 1997;Hamer et al. 2000), bacteria and viruses (Gollash et al. 1998). In general these organisms belong to the natural ecosystem in and around the port of origin but they might not be occurring naturally in the coastal waters and port of destination at the end of a ship's voyage. In hundreds of cases around the world, this has resulted in severe damage to the receiving ecosystem and to human health, because these non-native organisms developed into a plague. This often has a high impact on the ecosystem and can cause economical damage (Hoagland et al. 2002), as it results in a decrease of stocks of commercially valuable fish and shellfish species and occasionally outbreaks of diseases such as cholera (Ruiz et al. 2000; Drake et al. 2001). If action is not taken, the problem of invasive species will increase in an exponential manner for several reasons. Ships are getting larger, faster and the amount of traffic across the oceans is expected to increase rapidly during the coming decades, and therefore also the chance of non-indigenous organisms to have large enough numbers for settling and expanding. The problem of invasive species is considered as one of the 4 major threats of the world's oceans next to land-based marine pollution, overexploitation of living marine resources, and physical alteration/destruction of habitats To minimize these risks for the future, the International Maritime Organization (IMO) of the United Nations has adopted the Ballast Water Convention in 2004 (Anonymous 2005). The Convention states that finally all ships (>50,000 in number) should install proper ballast water treatment (BWT) equipment on board between 2009 and 2016. As a temporary and intermediate solution for the time being ship may reduce the risk of invasive species by performing ballast water exchange during their voyage when passing deep water (>200m depth and 200m from the coast) (Zhang F.Z & M Dickrnan1999). Ballast water exchange faces many problems as to feasibility, safety and efficacy for a large part of ships' voyages the required depth and/or distance to shore requirements are never met; BW exchange can affect the ships construction stability and in rough seas exchange is not possible because of the risk to ship and crew. Treatment of ballast water is therefore considered to be the best solution of reducing the risk of invasive species. During the recent years numerous solutions for treatment of ballast water have been mentioned and tested with the ultimate goal to reduce the amount of organisms in ballast water (Rigby & Taylor 2001). Recently a ballast water management system developed by Hyundai Group of Korea is firstly installed aboard a super crude ship. The company undertook the order from OSC company at 2008, which was the first time that installing a ballast water treatment equipment aboard a super crude ship. (http://twitter.com/yonhapcn) The ballast water treatment research in China is just at the experimental stage. To develop effective ballast water treatment system could play a great role in protecting Chinese even the whole world's ocean environment and reducing the risk of invasive species. First Institute of Oceanography, State Oceanic Administration conducted shipboard trials by using a UV disinfection system developed by Wuxi Brightsky Electronics Co., Ltd. The results of water quality and biology from the trials showed that it is a very effective ballast water treatment system.

2 Description of the facility

2.1 Introduction of the shipboard trials

Four trials were conducted aboard the LPG Carrier Huachang 8 (Figure 2.1) from July 2010 to Jan. 2011 for 6 months to test the efficacy of '*BSKY*^{TM} *BWMS*' (Ballast Water Treatment System). The trials were designed to document system performance under normal seagoing conditions. The flow rate during the trials was up to 250 m^3/h. The trials took place during the LPG Carrier's voyage schedule in South and North area of China seas. Trials consisted of determination of water quality parameters and a comparison of biological endpoints in treated and untreated ballast water samples, with reference to both IMO G8 guidelines and D-2 standard. Sampling procedures and endpoint determinations followed IMO G8 guidelines for shipboard trials.

2.2 Information of ship for trial

- Ship name: HuaChang 8
- Type: 3200 m^3 Liquefied Petroleum Gas Carrier（LPGC）
- Length overall: 97.0 m
- Length between perpendicular: 90.0 m
- Length of designed waterline: 92.7 m
- Molded breadth: 14.42 m
- Capacity of ballast pump：250 m^3/h
- Total volume of ballast tank：1574.38 Tons

Figure 2.1 Ship for trials: LPG Carrier Huachang 8

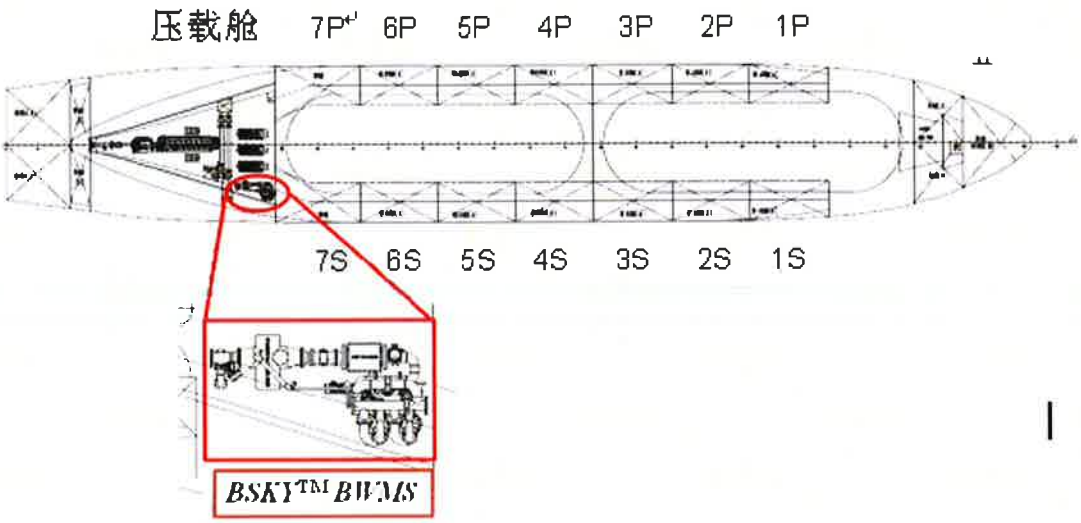


Figure 2.2 The location of *BSKY™ BWMS* inside the carrier

A series of matched tanks (3P, 3S, 5P, 5S) were used in every trial (Tabel 2.1). Tanks 3P and 3S were prepared for treated ballast water (treatment tank), while Tanks 5P and 5S for untreated water (control tank). At the beginning, 3P and 3S were filled with treated water and 5P and 5S filled with untreated water as the normal ballast water filling procedure (Figure 2.3). The 'treated first' protocol was designed to eliminate any possible false 'positives' through carry-over of untreated organisms in the ballast water system. For untreated samples, water was filled as the same path as the treated samples, except that the filter was by-passed and the UV unit was deactivated during the ballasting of the control tank.

Table 2.1 Name and volume of testing tanks

	Tank name	Number	Volume(m^3)
Treatment tank	3P	T1	121.2
	3S	T2	121.2
Control tank	5P	C2	111.5
	5S	C1	121.5

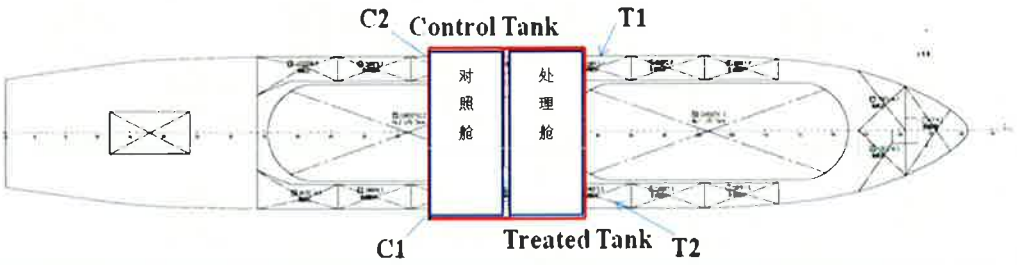


Figure 2.3 The location of the ballast tank for trials

2.3. Date of trials

Trials took place during the LPG Carrier's regular voyage. 4 trials were conducted and the detailed date information of the trials was shown in Table 2.2

Table 2.2 Shipboard Trials of *BSKY™ BWMS*

Trial	ballasting		de-ballasting	
	Site	Date	site	date
I	Huangdao of Qingdao	2010-7-19	Dongguan of Guangdong	2010-7-24
II	Dongguan of Guangdong	2010-7-24	Huangdao of Qingdao	2010-7-29
III	Zhoushan of Zhejiang	2010-8-10	Huangdao of Qingdao	2010-8-15
IV	Dongguan of Guangdong	2011-1-21	Huangdao of Qingdao	2011-1-28

2.4. Trial procedure

2.4.1 Sampling points and equipment

According to the requirement of G8, there were 2 sampling points (P1 and P2) in the shipboard trials.

P1: Before *BSKY™ BWMS*, Point A for influent water (control) during ballasting, Point C for untreated water during de-ballasting.

P2: After passing the *BSKY™ BWMS*, Point B for treated water during ballasting and de-ballasting.

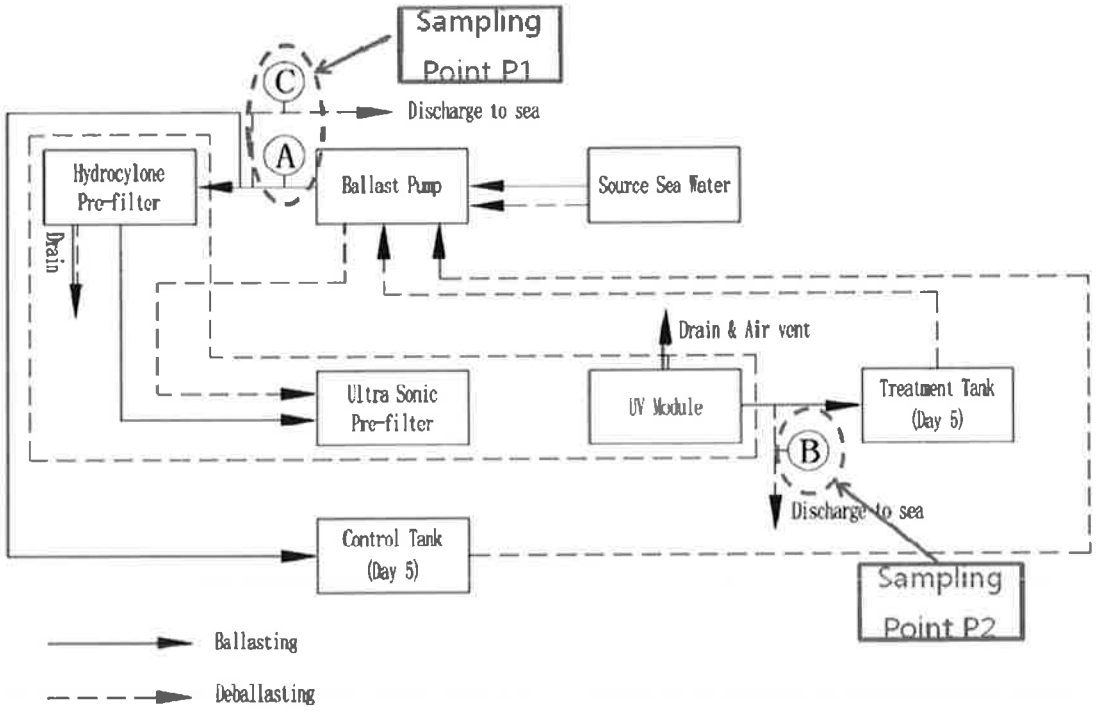


Figure 2.4 Sampling points

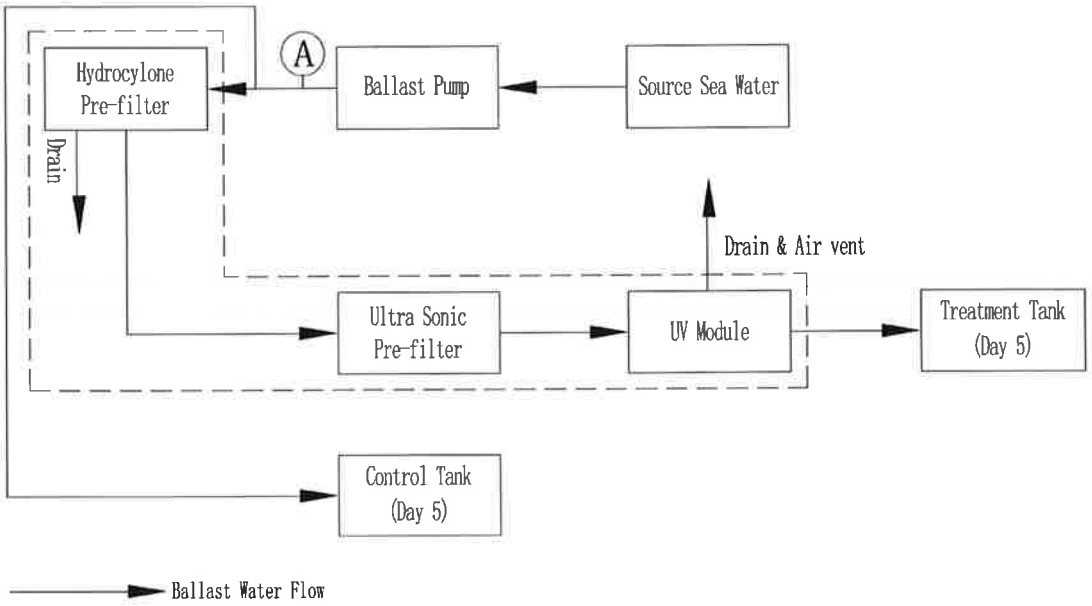


Figure 2.5 Procedure and sampling points at ballasting

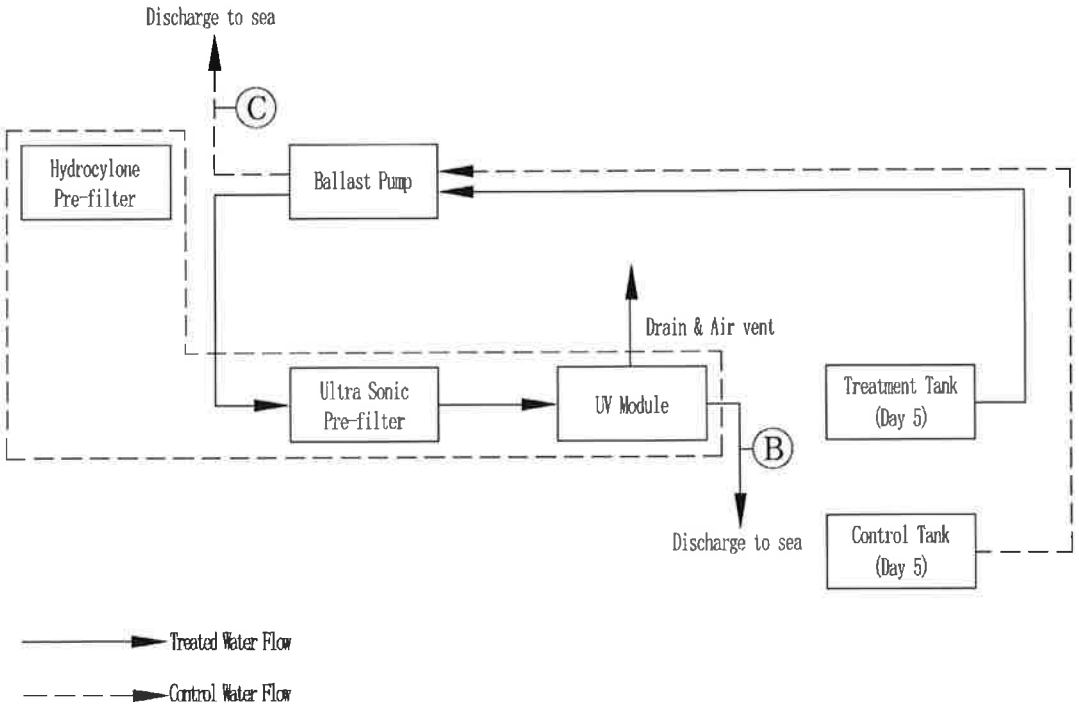


Figure 2.6 Procedure and sampling points at de-ballasting

The sampling device showed in Figure 2.7 was designed according to sampling specification ("California Standard", Topic 2, Part 3, Chapter 1, Article 4.7). A flow meter was fixed to the outlet of sampling device. The volume and rate of sample flow were 17.4 m^3/h and 2.2 m/s respectively. It took about 3.5 minutes to collect 1 m^3 water sample. The *BSKY*^{TM} *BWMS* was mounted inside of engine room, which was comparatively narrow and not so clean. The sample was collected outside of the engine room through a soft tube.

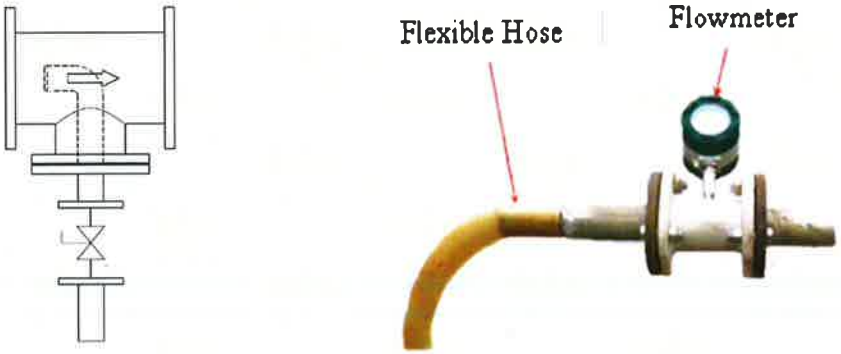


Figure 2.7 Sampling device and connection between flow meter and soft tube

2.4.2 Capacity of the *BSKY™ BWMS* and the trials

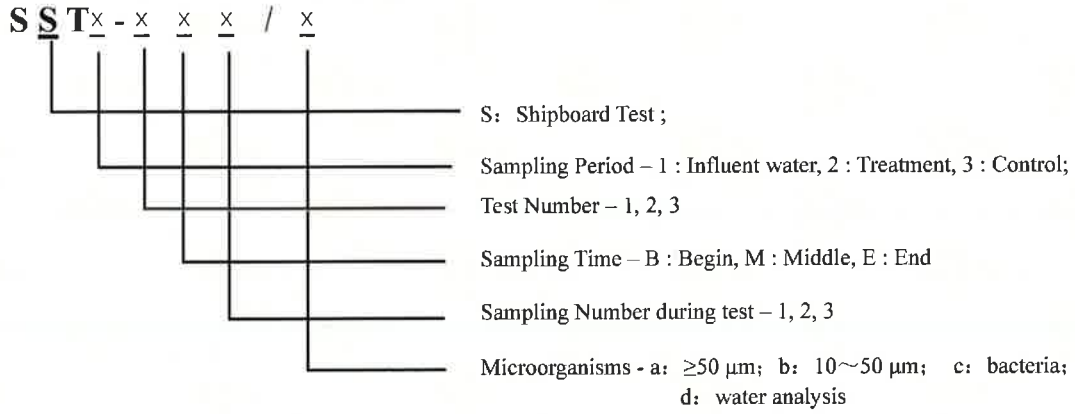
- Capacity: 250 $m^3/h \pm 10\ m^3/h$
- Treatment: twice treatment, treatment at ballasting and de-ballasting (not pass through hydrocyclone when de-ballast)
- Power consumption: < 30 kW
- Volume of water for trial: > 250 m^3 for each trial

2.4.3 Preparation before trials

For the trials, some reconstruction was carried out to mount the *BSKY™ BWMS* in the engine room. A pilot operation of the *BSKY™ BWMS* system was made on the land before it was transferred to the ship. Besides, a pre-trial run was conducted according to the procedure described in Section 2.4 to ensure the normal operation of the system. The overall process of trials was inspected by a supervisor from Nanjing Branch, China Classification Society. All the records of parameters measurement were valid only with the verification of the supervisor.

3 Methods of sampling and analyzing

3.1 Sample tag



3.2 Contents of the trial

The following parameters were measured in the trial :

- **Physical and chemical properties of test water:**
  Temperature, pH, Salinity, Turbidity (NTU), Dissolved oxygen, Dissolved organic carbon (DOC), Particulate organic carbon (POC) and Total suspended solids (TSS).
- **Biology:** Organisms (≥50 μm), Organisms (10 μm~50 μm) and photosynthetic activity in some trials.
- **Microbes :** Heterotrophic bacteria, *Escherichia coli,* Intestinal Enterococci, *Vibrio chlorerae.*

3.3 Sampling volume, sampling time and sampling method

Table 3.1 and table 3.2 show the sampling volume and time for various analyses respectively. Except for DO, samples for water quality testing were collected at discharge outlet directly with plastic buckets of 2.5 L. The samples were taken to the lab and well mixed, subsamples were then collected for water quality analysis or pre-treatments. For DO, samples were siphoned to brown bottles using gastight tubing, which was properly fitted to the sampling outlet of the ballast water simulating tanks. Samples for organisms (≥50 μm) were filtered through a net with diameter of 37 cm at opening and 1 meter length (Figure 3.1). Then the sample was transferred to a small bottle with a tag. Samples for the organisms between 10 μm ~50 μm were filtered through a net with diameter of 25 cm at opening and 25 cm length (Figure 3.2). 1 L of sample water was filtered and then transferred to small bottles with a tag. Samples for microbes were taken at the outlet directly in order to reduce the contamination of air. What's more, a delayed sampling was necessary to avoid the contamination from the inner of pipe. The sample bottles were treated under high temperature sterilization before sampling. Disposable gloves were worn and sterile operation was conducted as far as possible when sampling.

Figure 3.1 Sieve of 50 μm

Figure 3.2 Sieve of 10 μm

Table 3.1 Sampling volume and number of the Shipboard Trials of the *BSKY™ BWMS*

Type	Influent water at ballasting (C0)	Effluent water at de-ballasting of reference (C5)	Effluent water at de-ballasting of treated (T)
DO	150 ml ×1 ×3	150 ml×1×3	150 ml×3×3
NTU、pH、TSS、DOC、POC	2.5 L×1×3	2.5 L×1×3	2.5 L×3×3
Organisms≥ 50 μm	1 M^3 ×1×3	1 M^3×1×3	1 M^3×3×3
Organisms of 10 μm~50 μm	1 L×1×3	1 L×1×3	1 L×3×3
microbes	500 ml×1×3	500 ml×1×3	500 ml×3×3

Table 3.2 Sampling time of the Shipboard Trials of the *BSKY™ BWMS*

	Sample type	Number	Site	Date	Time (minute)
Trial 1	Influent water（C0）	3	Huangdao of Qingdao	2010-7-19	03:00~41:00
	Effluent water of treated tank after treatment（T）	9	Dongguan	2010-7-24	03:00~53:00
	Effluent water of reference tank when de-ballast（C5）	3	Dongguan	2010-7-24	03:00~41:00
Trial 2	Influent water (C0)	3	Dongguan	2010-7-24	03:00~41:00
	Effluent water of treated tank after treatment（T）	9	Huangdao of Qingdao	2010-7-29	03:00~53:00
	Effluent water of reference tank when de-ballast（C5）	3	Huangdao of Qingdao	2010-7-29	03:00~41:00
Trial 3	Influent water（C0）	3	Zhoushan	2010-8-10	03:00~41:00
	Effluent water of treated tank after treatment（T）	9	Huangdao of Qingdao	2010-8-15	03:00~53:00
	Effluent water of reference tank when de-ballast（C5）	3	Huangdao of Qingdao	2010-8-15	03:00~41:00
Trial 4	Influent water（C0）	3	Dongguan	2011-1-21	03:00~41:00
	Effluent water of treated tank after treatment（T）	9	Huangdao of Qingdao	2011-1-28	03:00~53:00
	Effluent water of reference tank when de-ballast（C5）	3	Huangdao of Qingdao	2011-1-28	03:00~41:00

Table 3.3 Sampling time for different kind of samples during the trials

type		Sampling time (min: sec)	Volume and number				Sample site
			Water quality	≥50μm	10μm~50μm	Heterotrophic bacteria	
A	Influent water when ballast(C0)	03:00	2.5 L	1 m^3	1 L	500 ml	A (P1)
		22:00	2.5 L	1 m^3	1 L	500 ml	
		41:00	2.5 L	1 m^3	1 L	500 ml	
B	Effluent water after second treatment (T)	03:00~22:00	2.5 L × 3	1 m^3 × 3	1 L × 3	500 ml × 3	B (P2)
		22:00~41:00	2.5 L × 3	1 m^3 × 3	1 L × 3	500 ml × 3	
		41:00~53:00	2.5 L × 3	1 m^3 × 3	1 L × 3	500 ml × 3	
C	Effluent water of reference tank when de-ballast (C5)	03:00	2.5 L	1 m^3	1 L	500 ml	C (P1)
		22:00	2.5 L	1 m^3	1 L	500 ml	
		41:00	2.5 L	1 m^3	1 L	500 ml	

3.4 The treatment and storage of samples

3.4.1 The treatment and storage of samples for water quality testing

The conditions of a normal office in the ship were suitable for the analysis usually. Samples can be analyzed in lab of ship once collected. The equipments for testing on-spot were taken to the ship in advance, debugging was necessary before testing to ensure its normality. All the samples should be analyzed or pre-treated within 6 h after collected. The temperature and salinity were determined with RBR directly when sampling, DO, pH and NTU were measured on-spot, samples for TSS, POC and DOC testing were stored in refrigerator in ship if colleted out of Qingdao ports, and taken back to Qingdao together with samples of de-ballasting in Huangdao using incubators or ice box, immediately saved at -20°C for further analysis when the samples arrived at Qingdao.

3.4.2 The treatment and storage of samples for biological analysis

After the samples were collected, analysis was immediately conducted. Viable organisms (≥50 μm and 10 μm ~50 μm) were respectively counted with a stereo microscope and an inverted microscope in the field lab. After the counting, samples were fixed (formalin was used for organisms ≥50 μm; and Lugol's solution for organisms 10 μm ~50 μm). After the whole test was completed, these samples were taken back to our lab in Qingdao to make further identification and counting. In order to promote the efficiency of determination, the efficiency of photosynthesis of phytoplankton were also measured in the two first trials. Samples for microbes testing must be collected with sterile operation, sample bottles were treated with high temperature pasteurization. Inoculation in the lab on site should be conducted immediately after sampling the samples would be cultivated in optimal conditions in incubator.

3.5 Methods and basis for analysis

3.5.1 Physical and chemical properties of test water:

1) **Temperature**: Using a RBR temperature sensor to measure the water temperature inside of the sample

bottles quickly.

2) **Salinity**: Using a RBR salinity sensor to measure the water salinity directly.

3) **pH**: pH-metric method, subsamples were measured on-site using a pH meter.

4) **NTU**: Spectrophotometric method. Subsamples were measured on-site using a spectrophotometer. Turbidity of subsamples was measured on-site using a spectrophotometer, determine the absorbance value at 660 nm wavelength.

5) **DO**: Winkler method. Samples were siphoned to special brown bottles using gastight tubing, which was properly fitted to the sampling outlet of the ballast water simulating tanks. These brown sample bottles were flushed with water volume more than 3 times of bottles' volume. The bottles were kept at dark containers until for further analysis. 1.0 ml of $MnCl_2$ and 1.0 ml of KI solutions were added to samples bottles before determining and inverted the bottles 20 times to mix the samples completely, then added 1.0 ml of H_2SO_4 sulotion to dissolve the precipitation, titrated with standard $Na_2S_2O_3$ solution and calculated the oxygen concentration expressed with mg/L.

6) **TSS**: Weight method pre-weighted glass fiber filters are used. Each filter was coded and stored in a clean petri dish. The filtered volume was dependent on the particle matter and concentration and type of organisms present in the water. The higher the total particle matter in the sample, the smaller was the volume that could be filtered before the filter clogs. Practical volumes were between 100 ml and 1000 ml per sample, after filtration the filter was rinsed with fresh water (Mili Q) to remove sea salt. Filters were dried overnight at 60 °C and allowed to cool in a vacuum desiccator before weighting. The total amount of suspended solids was calculated from the weight increase of the filter.

7) **POC**: High temperature combustion method, measured with an elemental analyzer. Water samples were filtered over pre-weighted glass fibre with 450°C combustion(the filtered volume was dependent on the particle load and concentration of organisms present in the water), and then measured with the elemental analyzer (ElementarVarioELIII, produced by German), three parallel samples for each sample.

8) **DOC**: High temperature combustion method, measured with TOC-V_{CPH} analyzer made in Japan for analysis. Samples for DOC (15 ml) were filtered through GF/F filters and sealed in pre-combusted glass ampoules after adding 50 μl of phosphoric acid (H_3PO_4), stored at -20 °C and taken back to our lab in Qingdao. Further measurement was conducted after samples were defrosted to room temperature. Standards were prepared with potassium hydrogen phthalate (Nacalao Tesque, Inc, Kioto, Japan). The mean concentration was calculated from triplicates of each sample. The average analytical precision of the instrument is < 3 %.

3.5.2 Biology

The majority of the large size fraction (≥50 μm) consists of zooplankton, while the majority of the small size fraction (10 μm ~50 μm) consists of phytoplankton. Samples were filtered over a 50 μm and a 10 μm sieve respectively (volume of filtered water is shown on Table 3.1). Then it was concentrated to 150 ml and poured into small plastic bottles, wash the sieve twice and transfer the flushing fluid to the plastic bottles together,

the samples for human pathogens analysis were taken in sterile sealed bottles.

1) **Organisms : ≥50 μm**

After sampling, identification and counting of viable organisms were taken with a stereo microscope before fixed. The whole sample for treated and control in discharge were determined, For influent water sample at ballasting, if the density of organisms is high, subsamples is suggested to be taken with a quantified sampling tube or a sample spliter which can separate the sample into 1/2, 1/5, 1/5 and 1/10 subsamples, and then analyzing one of the subsamples according to density. The examination of organism's activities was taken at 20×~160× magnification, give a record of identification and counting of viable individual. The states of organisms for this size fraction were easy to determine from their moving activity stimulated by light exposure careful stimulation with a fine needle under the stereomicroscope. When the counting of viable organisms was over, formaldehyde solution (the last concentration is 5%) was added to fix samples. A further identification and total amount of organisms was conducted after the samples were taken back to Qingdao. Then calculating the number using the unit ind/m^3.

The abundance of organisms:

$$C_B = \frac{N_B}{V}$$

where:

C_B — density of zooplankton per volume, unit (ind/m^3);

N_B — total number, unit(individual or cell) ;

V — the volume of filtering, uint (m^3).

2) **Organisms : 10 μm~ 50 μm:**

It is difficult to count all the organisms for 10 μm~50 μm, a practical method is to adjust the concentration of the cell to a constant value, after a proper mixing, take 1ml of well-distributed samples randomly and count with a counting chamber. The examination of organism's activities was taken with an invert microscope, give a record of identification and counting. For the untreated waters samples, we observe the activities of organisms before fixing and keep the record, usually we consider all of the organisms (10 μm~50 μm) viable according to the high yield value (Fv/Fm, usually > 0.4) which is known as a index of photosynthetic activity determined by Phyto-PAM, as a result, the counting for them was simple as described; for treated water samples, the identification and counting of viable organisms (10 μm~50 μm) were conducted immediately after the sample was collected, it was easy to identify the states of the flagellate-algae by its moving under the microscope, but for non-flagellate algae(especially for diatoms) this can be difficult, however, from the decreased yield value (<0.05), we believe that the physiological activity of viable organisms, if do exist, was greatly weakened although the concentration of Chlorophyll may be high, and the death rate could be calculated with the yield values of samples before and after treated. When the counting of viable organisms was over, formaldehyde solution (the last concentration is 1%) was added to fix samples. A further identification and total amount of organisms was conducted after the samples were taken back to Qingdao. Then calculating the number using the unit cell/L.

The expression is :

$$C = \frac{n \cdot V_1}{V_2 \cdot V_n}$$

Where:

C — organisms number per volume of sea water unit (cell/L);

N — counting number, unit (cell);

V_1 — sample volume after concentrated, unit (ml);

V_2 — sample filtered over small sieve, unit (L); (influent water of control 1L, treated water at discarge 10 L)

Vn — sample volume for counting, unit (ml). (we have two kind of counting chamber : 1ml and 0.5 ml).

3) The measuring method for photosynthetic activity

The photosynthetic activity (Fv/Fm) of phytoplankton with Phyto-PAM (Pulse-Amplitude Modulated fluorometer) was measured only in the two first trials. This instrument were used to other urvey project during the later trials. Otherwise, when the test ship arrived Qingdao harbor to ballast or de-ballast, some samples were stained by FDA-PI and observed in inverted fluorescence microscope.

(1) Sample collection

a) Water samples are collected, sample-rinsed Polyethylene bottles filled by hand

b) Samples are transported to the laboratory in ship and analyzed in 2 hours.

(2) Setup

a) Turn on computer and Phyto-PAM machine.

b) Turn off the Emitter-Detector Unit (ED).

c) Launch PhytoWin sofware program.

d) Check the Fluorescence values (data row F and Channels page). Values should be zero when the ED unit is off. A negligible reading of ± 8 is acceptable.

e) Click Report tab to bring up report page. Enter sample run information including date, run name and number, and collection info. Enter the Sample ID before running each sample.

f) Click Light Curve tab and turn on Blue, Green, and Brown in the Select box.

(3) Sample Analysis

a) The samples need a dark adaptation of 15 minutes.

b) Clean cuvette with deionized water and ethanol and dry completely, use Kimwipes to handle and clean the cuvette.

c) Transfer 3 ml of sample into the cuvette and place into ED unit. Keep ED unit cover on whenever possible. When removing the cover, be sure the ED unit is turned off.

d) Turn on the ED unit.

e) From the Channels page, press the Gain button to run automatic gain adjustment. It often takes 2 or 3 times to settle on a proper gain. Keep pressing Gain until the same reading comes up for a few consecutive times.

f) Turn off ED unit.

g) Remove cuvette, discard sample, and clean with deionized water.

h) Filter about 3 ml of sample throught a 0.2 μm filter into clean cuvette.

i) Place cuvette with filtrate into ED unit and turn it on, wait for Green Light at the bottom of the screen to come on, stable data measurement.

j) Click the Zoff button to set an automatic baseline adjustment for the sample.

k) Turn off ED unit.

l) Remove cuvette and discard filtrate.

m) Transfer 3 ml of sample (unfiltrate) into the cuvette.

n) Place in ED unit and turn it on. Wait for Green Light.

o) Click Start One button and wait for measurement. Wait for Green Light.

p) Click Chl(Fo) button and wait for measurement. Wait for Green Light.

q) Go to Light Curve page by clicking the tab. When light at bottom of page is green, click Light Curve button to initiate light curve. When curve is finished, click Fit button.

r) Go to Options Menu at top of page, and select Light Curve Fit Parameters.

s) Copy the data to a Pam Data Sheet.

t) Go to the File Menu and Save the report in the appropriate folder.

u) Return to the Channels page, click New Record button and turn off the Zoff.some.

4) The staining method of 10 μm~ 50 μm organisms by FDA-PI

Subsamples will be stored with no light and transported to laboratory. In the laboratory, FDA will first be added to the sample; after fully mixing PI will be added to the sample. Under blue light (Max wavelength: 495 nm), alive cell are stained to be bright green and dead cell are stained to be red. Quantity of organisms will be observed and counted by using fluorescence microscope.

Cell staining. The supernatant was discarded and the cell stained with FDA-PI.

A stock solution of fluorescein diacetate (Sigma) was prepared by dissolving 5 mg/ml in acetone. FDA working solution was freshly prepared by adding 0.04 ml of stock to 10 ml of Dulbeceo's phosphate buffered saline(DPBS).To stain with FDA-PI 0.1 ml (2 μg) of FDA working solution and 0.03 ml (0.6 μg) resuspended cells, cells were stained for 3 min at room temperature then placed on ice.

3.5.3 Analysis of human pathogens

Inoculation should be taken immediately, then sealed the samples with complete plastic bag and took back to our lab in Qingdao, cultured under optimal temperature condition and determine the number of colony forming units (cfu's) according to international standard.

1) Heterotrophic bacteria: plate method

Principles: After incubation of a sample, the dispersed bacteria will develop into isolated colonies. A visible colony on solid medium represents one bacterial cell. The number of heterotrophic bacteria is obtained by counting the number of colonies. The key of this technique is to disperse the heterotrophic bacteria completely and to dilute bacterial sample to several solutions with different concentration. Small volume of diluted solution (containing 100 cells to 200 cells or less) is spread evenly over the surface of the solid medium.

Procedures: 1 ml Tween solution was added to 100 ml sample. The sample was well mixed to separate the

organisms and kept them separated. Take 1ml of the sample with a sterile pipette to a test tube filled with 9 ml of disinfected sea water. After a thorough mixing, 0.1ml of solution was taken and inoculated on the surface of solid medium (2216E) in a Petri dish. Then it was spread evenly with a sterile, L-shaped glass rod. The dish was incubated at 25 ℃ for 7d, and then it was taken out for counting the number of colonies.

2) *vibrio cholerae*: plate method

The total amount of vibrio is one of the important parameter for indicating water pollution levels of human pathogens. TCBS selective medium is chosen to examine the amount of vibrio. After the inoculation to the medium in a dish, the dish was incubated for a certain time under optimal conditions. Then the vibrio colonies were counted.

Procedure: 1ml of sample was pipette with sterile operation and inoculated into a test tube with BTB medium solution. It was incubated for 18h at 37 ℃. The bacterial solution shown a positive reaction was taken and lined on TCBS plate, which will be cultivated for 18h at 37℃. The colonies with green, blue-green and yellow color will be inoculated on CPA plate with tilted surface. Series experiments including the gram stain, oxidase testing, motility and 0/139 sensibility testing of vibriocin for the bacterial colonies separated were conducted. Check the MPN tube number of the bacterial strain with characteristics of vibrio and calculate the number according to the MPN Table.

3) *Escherichia coli*: membrane filter technique

The water sample was filtered through a membrane filter. After filtration, the heterotrophic bacteria were on the membrane. Then the filter was placed on a selective solid medium and there should be no entrapment of air. After incubation, the Escherichia coli colonies on the membrane were identified and counted. The number of Escherichia coli per liter sea water was then worked out.

procedure: 100 ml of sample water was filtered through an acetates membrane with pore diameter of 0.2μm. After filtration, the heterotrophic bacteria were remained on membrane. The membrane was placed on the surface of a solid medium (M-TEC) without any entrapment of air. After 0.5 h cultivation with the plate inverted in an incubator at 37 ℃, it was transferred to another incubator with 44 ℃ for a continuous cultivation of 18 h-24 h. The Escherichia coli colonies on the membrane were counted and identified. The number of Escherichia coli per liter sea water was then worked out.

4) *Intestinal enterococci*: membrane filter technique

PSE agar plate with selective culture medium is chosen to test the total number of intestinal enterococci. After inoculation, the plate is cultivated in an incubator at 37 ℃for a certain time. The bacterial colonies with characteristics of intestinal enterococci were counted. The colonies may be isolated and purified for further identification. The procedure is the same as that for Escherichia coli.

3.5.4 Guidelines and Specifications followed

1) Guidelines for approval of ballast water management systems (G8) Resolution MEPC. 174（58）

   According to the D-2 Standard of the IMO/MEPC Convention of 2004 (Anonymous 2005) ships that meet the requirements of the Convention by meeting the ballast water performance standard must discharge:

(1) Less than 10 viable organisms per cubic metre greater than or equal to 50 micrometers in minimum dimension;

(2) Less than 10 viable organisms less than 50 micrometers in minimum dimension and greater than or equal to 10 micrometers in minimum dimension ;

(3) Less than the following concentrations of indicator microbes, as a human health standard:

① Toxicogenic *Vibrio chlorerae* (serotypes O1 and O139) with less than 1 colony forming unit (CFU) per 100 milliliters or less than 1 CFU per 1 gramme (wet weight) of zooplankton samples:

② *Escherichia coli* less than 250 CFU 100 milliliters;

③ *Intestinal Enterococci* less than 100 CFU per 100 milliliters.

2) *BSKY*^{TM} *BWMS* ultraviolet light disinfection system approval of testing program

3) Part 5 of the specification for oceanographic survey-chemistry (GB/T12763.5-2007)

4) Part 6 of the specification for oceanographic survey-biology (GB/T12763.6-2007)

5) The specification for marine monitoring-Part 7: water quality monitoring and analysis (GB17378.4-2007)

6) The specification for marine monitoring-Part 7: Ecological survey for offshore pollution and biological monitoring (GB17378.7-2007)

7) Manual on harmful marine microalgae, G.M Hallegraeff, D.M. Anderson and A.D. Cambella. Intergovernmental oceanographic commission. Manuals and Guides 33. 1995.Paris.

Table 3.4 Summary of parameters, method, sensibility and guidelines of the test

Parameters	Unit	MDL	Method of analysis	Sensibility	Guideline
temperature	℃	NA	RBR temperature sensor	0.1℃	The specification for oceanographic survey
Salinity	PSU	1.0	RBR salinity sensor	0.1PSU～0.2 PSU	The specification for oceanographic survey
pH	pH	0.0	pH meter	0.01 pH	The specification for marine monitoring
DO	mg/L	0.1 0.2	winkler method	0.05 mg/L	The specification for marine monitoring, specification for oceanographic survey
NTU	NTU	0.1	spectrophotometric method	0.1 NTU	The specification for oceanographic survey
DOC	mg/L	0.36	high temperature combustion method		The specification for marine monitoring
POC	mg/L	0.1	high temperature combustion method		The specification for marine monitoring
TSS	mg/L	1.0	Weight method		The specification for oceanographic survey
Organisms ≥50 μm	ind/ml	1.0	Filtered and condensed with 50 μm sieve， count with microscope		The specification for oceanographic survey
Organisms 10 μm～50 μm	cell/ml	1.0	Filtered and condensed with 10 μm sieve， count with invert microscope		Hallegraeff.G.M ,D.M. Anderson and A.D. Cambella
heterotrophic bacteria	CFU/ml	1.0	Plate method		The specification for marine monitoring
E.coli	CFU/ml	1.0	Filter membrane method		The specification for marine monitoring
Intestinal enterococci	CFU/ml	1.0	Fecal Streptococcus and Enterococcus group		Standard Method 9230 or MM-FS-CNJ-0351 or ISO4833-2003
Vibrio cholerae	CFU/ml	1.0	Plate method		The specification for marine monitoring

3.6 Quantity control

3.6.1 Measures for quality assurance

3.6.1.1 Measures of sampling at test site for quality assurance

All samples were collected on the test site. The water samples were distributed into bottles with tags or labels. To avoid or reduce contamination, the sample bottles were cleaned with hydrochloric acid (samples for pH measurement were not included), then washed with pure water at least twice. Before sampling, the bottles were washed twice again with the sea water of test site. The sample bottles for microbes were autoclaved. The culture medium for microbes incubation were prepared in the lab. Before the test, they were disinfected at test site. Small plankton nets with 50μm and 10μm mesh size were used for filtering the organisms (≥50μm) and the organisms (10 μm~50 μm) respectively. After that, the samples were concentrated and transferred into small sample bottles.

3.6.1.2 Measures of storage and transport of samples for quality assurance

During the operations of filtration and distribution of samples, measures against contamination were adopted. When collecting sample for POC, DOC and microbes, it is required to wear gloves. The samples, such as DOC, and POC can not be analyzed at test site. They were stored under frozen after pre-treatment. During transportation, they were in a container with dry ice. Plankton samples were fixed and the sample bottles were sealed. Then they were taken back to lab in Qingdao for further analysis.

3.6.2 Quantity control

3.6.2.1 Quantity control of analysis

- All analytical equipments used have to meet the requirements of the test.
- The samples need to be carefully checked prior to analysis. That is the samples are kept well. The inside and outside labels coincide with the records taken during the test.
- Equipment must be still in normal condition after the analysis.
- When abnormal results were suspected, the causes should be found out in time and explanation and correction should be made. There is a need to repeat the analysis if necessary.
- Except for postgraduate students, all of the staff conducting measurements and analyses should be qualified to do marine environmental monitoring with certificate. The students have to take in special technical training and their work will be supervised.

3.6.2.2 Quantity control during the trials

- A technical introduction and work allocation about the test will be given to all participating staff. Everyone must clearly understand his/her responsibility for work and results.
- The equipments should be checked as soon as they were in the test site to see if everything is OK. There will be another check when the equipment was set up to see if it runs normally. The equipment will be calibrated if necessary. All these activities will be recorded.
- All samplings and analyses follow relevant valid version of standards, guidelines and specifications.
- The equipment will be checked when all work were finished. It should be in normal condition.

- If the analysis was interrupted or some changes of sampling or analysis have to be made, it should be reported first to the leader of the test. The work could be continued only if it was approved.

3.6.2.3 Quantity control of equipments used

All the equipments were examined by legal authority designated by state. The allowance should be still valid. If the equipment needs only self examination, it should be examined by relevant experts prior to the test.

3.6.3 The raw records

1) The raw records reflect the exact results of sampling and analyses. Any change and deletion of them is strictly prohibited. The raw records of sampling have to be checked by the supervisor from Nanjing Branch, China Classification Society with his/her signature at the test site.
2) Tables with unified format should be used for taking the raw records. The use of pencil was not allowed except there is a special definition. The Tables should be filled out completely with signature of the analyzer and proofreader.
3) The determination of significant digits and data processing of the raw data should strictly follow the relevant definition in the National standards of China --The Specification for Oceanographic Survey (GB/T12763-2008)and the Specification for Marine Monitoring (GB17378.7-2007).

4 Results

4.1 Physical parameters

The temperature and salinity of water samples in the first trial, when ballasting in Qingdao were 23.7 ℃and 31.4 PSU, respectively, while at de-ballasting in Dongguan, the temperature raised by 5.6 ℃, the salinity reduced slightly. When ballasting at Dongguan in the second trial, the temperature was up to 29.9 ℃, while the salinity which slightly increased (1.3 PSU) when de-ballasting back to Qingdao was only 0.8 PSU because of the ebbing. The temperature reduced by 4 ℃ at de-ballasting in Qingdao. The ballasting of the third trial took place at an anchorage of Zhoushan, Zhejiang province where the salinity and temperature of water samples were 27.8 PSU and 25.8 ℃, respectively, which not changed apparently. The last trial ballasting in Dongguan showed a temperature of 15.4 ℃ on average, the salinity was 6.0 PSU higher compared to that in summer of this sea area, which may be caused by tiding when ballasting. When the ship went back to Qingdao, the temperature declined more than 10 ℃.

Table 4.1 Salinity and temperature of water during the Shipboard Trials of the *BSKY™ BWMS*

Trial	Ballast				De-ballast			
	Site	Date	Salinity (PSU)	Temp. (℃)	Site	Date	Salinity (PSU)	Temp. (℃)
I	Huangdao of Qingdao	2010-7-19	31.4	23.7	Dongguan	2010-7-24	31.0	29.3
II	Dongguan	2010-7-24	0.8	29.9	Huangdao of Qingdao	2010-7-29	1.3	25.8
III	Zhoushan	2010-8-10	27.8	25.8	Huangdao of Qingdao	2010-8-15	27.6	26.1
IV	Dongguan	2011-1-21	6.7	15.4	Huangdao of Qingdao	2011-1-28	5.8	4.14

4.2 Chemical parameters

A summary of the results of the chemical parameters is presented in Table 4.2 for the four trials, which shows that it was greatly different between trials in the water quality. The NTU in Qingdao port was the lowest, while the water condition in Dongguan was identical to fresh water, and the NTU was the highest. The DO closely related to temperature was lowest in Dongguan during 3 trials in summer the average value was only 4.19 mg/L. The lowest pH took place in summer and winter trials in Dongguan, was 7.69 and 7.35, respectively. While in Qingdao and Zhoushan, the pH was nearly 8.00. The concentration of TSS fluctuated from 1.63 to 54.20 mg/L. The POC was relatively low (less than 1.00 mg/L) except that the value in Dongguan sea area was over 2.00 mg/L in summer, especially the POC of de-ballasting water was even less than 0.50 mg/L, no matter it was in the treated tank or the reference tank. On the other hand, the concentration of DOC was apparently higher than POC except in summer in Dongguan, the concentration of DOC in Qingdao was nearly 7 times of POC and in Zhoushan it was about 4 times, while it was 1 time and 10 times in summer and winter in Dongguan, respectively.

Table 4.2 Testing results of NTU, DO, pH, TSS, POC and DOC concentrations during the Shipboard Trials of the *BSKY*^{TM} *BWMS*

parameter	Influent water		Effluent water(day 5)			
	Average	SD	Effluent water of the reference		Effluent water of treated	
			Average	SD	Average	SD
Trial 1 Qingdao (ballast) – Dongguan (de-ballast) 2010.7.19 - 2010.7.24						
NTU	2.40	0.20	4.74	0.69	3.17	0.19
DO (mg/L)	7.23	0.13	6.71	0.17	6.38	0.17
pH	7.90	0.02	7.98	0.03	7.94	0.02
TSS (mg/L)	27.6	5.07	19.05	1.54	10.50	10.93
POC (mg/L)	0.50	0.11	0.15	0.01	0.23	0.07
DOC (mg/L)	3.55	2.13	1.87	0.25	1.89	0.40
Trial 2 Dongguan (ballast) – Qingdao (de-ballast) 2010.7.24 - 2010.7.29.						
NTU	26.29	2.87	1.77	0.15	1.95	0.24
DO (mg/L)	4.19	0.19	5.10	0.53	4.33	0.15
pH	7.69	0.02	7.02	0.03	7.02	0.04
TSS (mg/L)	42.00	7.69	8.67	2.60	5.71	2.62
POC (mg/L)	2.11	0.09	0.41	0.02	0.34	0.02
DOC (mg/L)	2.28	0.80	1.86	0.27	2.26	0.13
Trial 3 Zhoushan (ballast) – Qingdao (de-ballast) 2010.8.10 - 2010.8.15						
NTU	14.08	2.40	0.95	0.42	0.50	0.20
DO (mg/L)	6.36	0.11	6.40	0.07	6.39	0.11
pH	7.93	0.01	7.88	0.04	7.91	0.02
TSS (mg/L)	47.17	8.16	9.25	1.74	9.44	0.98
POC (mg/L)	0.35	0.04	0.12	0.01	0.12	0.02
DOC (mg/L)	1.40	0.31	1.66	0.50	2.80	2.71
Trial 4 Dongguan (ballast) – Qingdao (de-ballast) 2011.1.21 - 2011.1.28						
NTU	9.94	0.39	4.25	3.65	1.50	0.01
DO (mg/L)	6.29	0.04	6.44	0.36	6.32	0.10
pH	7.35	0.01	7.59	0.04	7.68	0.07
TSS (mg/L)	47.70	9.99	3.23	1.41	15.64	19.99
POC (mg/L)	0.33	0.06	0.20	0.05	0.29	0.18
DOC (mg/L)	3.91	0.47	2.50	0.11	2.83	0.16

4.3 Organisms≥50 μm

The organisms (≥50 μm) was referred to zooplankton for the trials on shipboard, the abundance of this size fraction was highest in Qingdao sea area, where the value was 4.60×10^4 ind/m^3 on average, while in Zhoushan it was the lowest (3.77×10^3 ind/m^3), one order of magnitude lower than that in Qingdao, at last, the abundance in Dongguan was 6.71×10^3 ind/m^3 and 1.62×10^4 ind/m^3 in summer and winter, respectively. Compared with the water at ballasting in the reference tank, the abundance of organisms (≥50 μm) at de-ballasting was clearly decreased, which may be caused by the difference of water condition after 5 days travel, especially the change of temperature between Qingdao and Dongguan was 5.6 °C and 11.3 °C in summer and winter, respectively, which may result in the death of organisms. As is demonstrated in Table 4.3, the abundance of organisms was 1.39×10^2 ind/m^3 on average at de-ballasting of the reference tank in the first trial, only 1/300 of that at ballasting. In the same way, during the second trial in winter, the abundance of organisms at de-ballasting was 1/30 of that at ballasting; the third trial showed that the temperature differed less than 1 °C between Zhoushan and Qingdao and the abundance of organisms was only reduced by 24 % (3.77×10^3 ind/m^3 when ballast and 2.87×10^3 ind/m^3 when de-ballast). At last, the abundance of organisms when ballast in Dongguan was 91% higher than that when de-ballast in Qingdao during the last trial of reference tank. The results of treated tanks of different trials showed that only one sample was positive for the viable organisms, which was presented on the second trial though only 1 viable organism was observed, the activity of this kind of zooplankton (*Cyclopoida*) was so little, it would be dead within several hours. No viable organisms were observed in the other 3 trials, which met the D-2 standard and the G8 requirement well.

Table 4.3 Abundance of viable organisms (≥50 μm) for water samples when ballast and de-ballast during the Shipboard Trials of the *BSKY^{TM} BWMS*

Trial 1 Qingdao (ballast) – Dongguan (de-ballast) 2010.7.19 - 2010.7.24			
Parameter	Influent water (C0)	Effluent water(day 5)	
	Average (n=3)	Reference (C-5)	Treated (T)
		Average (n=3)	Average (n=9)
Total density (ind/m^3)	46,010	139	0
Range	21,068~58,922	85~204	
Trial 2 Dongguan (ballast) – Qingdao (de-ballast) 2010.7.24 - 2010.7.29.			
Total density (ind/m^3)	6,711	3,505	0
Range	1,693~16,101	3,040~3,975	
Trial 3 Zhoushan (ballast) – Qingdao (de-ballast) 2010.8.10 - 2010.8.15			
Total density (ind/m^3)	3,767	2,867.7	0
Range	3,434~3,933	1,543~3,600	
Trial 4 Dongguan (ballast) – Qingdao (de-ballast) 2011.1.21 - 2011.1.28			
Total density (ind/m^3)	16,173	560	0
Range	14,840~17,440	260~861	

4.4 Organisms (10 μm~50 μm)

This size fraction was mainly composed of plankton and protozoa although the community structure was various in different sea areas (Table 4.4), for example, in Qingdao the dominant species were large

individuals: *Coscinodiscus asteromphalus*, *Ceratium lineatum* and *Chaetoceros curvisetus*, while in Zhoushan the species mainly included *Skeletonema costatum*, *Pseudonitzschia pungens*, *Rhizosolenia setigera*; besides, Jiufeng port of Dongguan was in estuary of the Pearl River where the plankton community were all fresh water species, the dominant species was *Melosira granulate*, *Actinastrum hantschii*, *Pediastrum duplex* and *Scenedesums* sp., what's more, when ballast in winter it was a rising tide which resulted in a presence of both fresh water species and sea species, but the fresh species was still in dominant, mainly include: *Microspora stagnorum*, *Coscinodiscus* spp., *Hormidium* spp. and *Paralia sulcata*.

The influent water in reference tank at ballasting contained a low density of plankton in Qingdao and Zhoushan sea area, mostly fluctuated above 102 cell/ml, while the abundance in Dongguan was clearly high (two orders of magnitude higher than that in Qingdao and Zhoushan), however, 5 days later, the abundance of all trials declined, about 60 % in the first trial, 70 % in the third trial and 66 % in the last trial, the greatest fluctuation was presented in the second trial in which the abundance was declined by more than 80%, besides, the number of kind was relatively decreased too. The viable plankton was only observed in the second trial, the density was 0.04 cell/ml on average (Table 4.5). In short, the density of viable organisms during 4 trials was only 0.01 cell/ml on average which met the D-2 standard, G8 requirement and American standard.

Table 4.4 Dominant species of plankton (10 μm~50 μm) in the Shipboard Trials of the *BSKY™ BWMS*

Species	community	Qingdao (summer)	Zhoushan (summer)	Dongguan (summer)	Dongguan (winter)
Ceratium lineatum	dinoflagella	++++			
Chaetoceros curvisetus	diatom	+++			
Coscinodiscus asteromphalus	diatom	+++			
Skeletonema costatum	diatom	++	++++		
Pseudonitzschia pungens	diatom		+++		
Rhizosolenia setigera	diatom		+++		
Coscinodiscus jonesianus	diatom		++		
Melosira granulata	diatom			++++	
Actinastrum hantschii	Chlorophyta			++++	
Pediastrum duplex	Chlorophyta			+++	
Scenedesums dimorphus	Chlorophyta			++	
Cyclotella sp.	diatom			++	
Coscinodiscus spp.	diatom				+++
Microspora stagnorum	Chlorophyta				+++
Hormidium spp.	Chlorophyta				++
Paralia sulcata	diatom				+

In order to take a further examination of the treatment effect, we also tested the photosynthetic activity (Fv/Fm) of phytoplankton directly with Phyto-PAM in the first and second trial, the results were shown on Table 4.6, which demonstrated that: in the first trial, the photosynthetic activity of phytoplankton for the influent water and effluent water of the reference tank was 0.57 and 0.19, respectively, while for the effluent

water of treated tank, the value was only 0.01, and five water samples were even negative for photosynthetic activity, which means that the plankton nearly lost their photosynthetic activity after treatment; in the second trials, photosynthetic efficiency was higher than that in the first trial, it also inclined slightly when de-ballasting 5 days later, averaged to 0.67, which shows a different trend with the density of plankton that did decline clearly when de-ballasting (Table 4.6), the average value of photosynthetic efficiency in treated tank was 0.02, a little higher than that in the first trial, it was still need a further study about whether it was caused by a high NTU or high TSS. All in all, the efficiency of treatment was up to 97%.

Table 4.5 Abundance of viable organisms (10 μm ~ 50 μm) for water samples when ballast and de-ballast during the Shipboard Trials of the *BSKY™ BWMS*

Trial 1 Qingdao (ballast) – Dongguan (de-ballast) 2010.7.19 - 2010.7.24			
Parameter	Influent water (C0)	Effluent water(day 5)	
	Average (n=3)	Reference (C-5)	Treated (T)
		Average (n=3)	Average (n=9)
Total density (ind/ml)	247.5	98.7	0
Range	119~333	63~161.2	
Trial 2 Dongguan (ballast) – Qingdao (de-ballast) 2010.7.24 - 2010.7.29.			
Total density (ind/ml)	5,301	928	0.009
Range	2,710~9,323	282~1,584	0~0.26
Trial 3 Zhoushan (ballast) – Qingdao (de-ballast) 2010.8.10 - 2010.8.15			
Total density (ind/ml)	403	118	0
Range	254~548	82~142	
Trial 4 Dongguan (ballast) – Qingdao (de-ballast) 2011.1.21 - 2011.1.28			
Total density (ind/ml)	4,494	1,386	0
Range	3,940~5,301	553~2,834	

Table 4.6 Photosynthetic efficiency（Fv/Fm）for water samples when ballast and de-ballast during the Shipboard Trials of the *BSKY™ BWMS*

Fv/Fm		Influent water (C0)	Effluent water (day 5)	
			Reference (C-5)	Treated (T)
Trial1	Average	0.57(n=9)	0.19(n=3)	0.01(n=9)
	SD	0.01	0.02	0.01
Trial2	Average	0.67 (n=3)	0.65 (n=3)	0.02(n=9)
	SD	0.01	0.01	0.01

4.5 Heterotrophic bacteria and Human pathogens

Table 4.7 shows the results of heterotrophic bacteria and human pathogens when ballast and de-ballast during the Shipboard Trials of the *BSKY™ BWMS*, the number of heterotrophic bacteria colonies of reference tank when ballasting in all trials was near 10^6 CFU/100ml, however, when the water was de-ballasted 5 days later, the number was declined to the same order: the separate number for each trial was 2.89 CFU/100ml, 12.00

CFU/100ml, 6.22 CFU/100ml and 0 CFU/100ml. There were none viable Vibrio cholerae observed after an incubation of sample bottles to the treated water from the 4 trials. The number of Intestinal *enterococci* and *Escherichia coli* colonies were all less than 1 CFU/100ml after treated.

Table 4.7 Average number of heterotrophic bacteria and human pathogens for water samples when ballast and de-ballast during the Shipboard Trials of the *BSKY™ BWMS*

Trial 1 Qingdao (ballast) – Dongguan (de-ballast) 2010.7.19 - 2010.7.24			
Parameter (CFU/100ml)	Influent water(C0)	Effluent water(day 5)	
	Average (n=3)	Reference (C5)	Treated (T)
		Average (n=3)	Average (n=9)
Heterotrophic bacteria	3.13×10^6	2.10×10^6	2.89
Vibrio cholerae	5.70×10^2	7.30×10^2	0
Escherichia coli	26.70×10^2	13.70×10^2	0.56
Intestinal enterococci	15.30×10^2	5.00×10^2	0.44
Trial 2 Dongguan (ballast) – Qingdao (de-ballast) 2010.7.24 - 2010.7.29.			
Heterotrophic bacteria	2.59×10^6	2.42×10^6	12.00
Vibrio cholerae	6.33×10^2	6.70×10^2	0
Escherichia coli	30.67×10^2	16.00×10^2	0.67
Intestinal enterococci	16.00×10^2	11.30×10^2	0.56
Trial 3 Zhoushan (ballast) – Qingdao (de-ballast) 2010.8.10 - 2010.8.15			
Heterotrophic bacteria	1.37×10^6	1.19×10^6	6.22
Vibrio cholerae	2.30×10^2	1.6×10^2	0
Escherichia coli	25.00×10^2	16.3×10^2	0.33
Intestinal enterococci	10.33×10^2	7.00×10^2	0.22
Trial 4 Dongguan (ballast) – Qingdao (de-ballast) 2011.1.21 - 2011.1.28			
Heterotrophic bacteria	1.33×10^5	1.99×10^5	0
Vibrio cholerae	7.70×10^2	9.27×10^2	0
Escherichia coli	3.90×10^3	4.46×10^3	0
Intestinal enterococci	10.70×10^2	8.70×10^2	0

5 Conclusion and evaluation of results

The Shipboard Trials of *BSKY* produced by Wuxi Brightsky Electronics Co., Ltd. was conducted on Hua Chang 8 Liquefied petroleum Gas Carrier for 4 trials though the summer and winter, which complied with the time requirements of G8. According to the testing results and the reference of G8 and D-2 standard (Table 5.1), the conclusion was made as follows:

1) The organism densities for different size fraction of the 4 Shipboard Trials were various from each other: for the large size fraction (≥50 μm), the density fluctuated between 1.69×10^3 ind/m^3 and 5.89×10^4 ind/m^3 ; while the value for small size fraction (10 μm ~ 50 μm) was from 1.19×10^2 cell/ml to 9.32×10^3 cell/ml. All the densities were more than 10 times of the greatest number defined by D-2 standard, as a result, all of the 4 trials were valid.

2) Viable organisms (≥50 μm) were only observed in one sample of the second trial for the effluent water of treated tank during the 4 trials, but the living activity was very weak, it may be die within several hours, no survivals were observed for the other three trials. All the results met the D-2 standard and G8 well.

3) For the viable plankton (10 μm ~ 50 μm), two samples of the second trials were proved positive for that, the density was 0.04 cell/ml on average, none survivals were observed for the other three trials, the average density for the 4 trials was only 0.01 cell/ml, which met the D-2 standard and G8 requirement.

4) The number of heterotrophic bacteria for each trial was 2.89 CFU/100ml, 12.00 CFU/100ml, 6.22 CFU/100ml and 0 CFU/100ml on average, respectively. There were none viable *Vibrio cholerae* observed after an incubation of sample bottles to the treated water from the 4 trials. The number of Intestinal enterococci and *Escherichia coli* colonies were all less than 1 CFU/100ml after treated which met the D-2 standard and G8 requirements.

All in all, according to the results of the Shipboard Trials of the *BSKY*^{TM} *BWMS*, although several samples of shipboard trial had a slightly higher density of viable organisms than that in the land-based test, the removal effect to different size fraction of organisms still met the D-2 standard, and the efficiency of treatment was over 99.9%.

Table 5.1 Comparison of Shipboard Trials results of *BSKY*^{TM} *BWMS* with D-2 standard and Guideline 8

	Parameters	D-2 standard and Guideline 8		Testing results			Assessment
		Influent water	Treated water	Influent water	Effluent water of control	Effluent water of treated	
Trial 1	≥50 μm (ind/m^3)	>100	<10	4.60×10^4	1.39×10^2	no living organism	meet the D-2 standard and Guideline 8
	10 μm ~50 μm (cell/ml)	>100	<10	2.47×10^2	98.70	no living organism	meet the D-2 standard and Guideline 8
	<10μm -Bacteria(CFU/100ml)	$\geq10^4$	No defined	3.13×10^6	2.10×10^6	2.89	meet the D-2 standard and Guideline 8
	Escherichia coli(CFU/100ml)	>2500	<250	26.70×10^2	13.70×10^2	0.56	meet the D-2 standard and Guideline 8
	Intestinal Enterococci(CFU/100ml)	>1000	<100	15.30×10^2	5.00×10^2	0.44	meet the D-2 standard and Guideline 8
	Vibrio choleerae(CFU/100ml)	>10	<1	5.70×10^2	7.30×10^2	0	meet the D-2 standard and Guideline 8
Trial 2	≥50 μm (ind/m^3)	>100	<10	6.71×10^3	3.51×10^2	no living organism	meet the D-2 standard and Guideline 8
	10 μm ~50 μm (cell/ml)	>100	<10	5.30×10^3	9.28×10^2	0.009	meet the D-2 standard and Guideline 8
	<10μm -Bacteria(CFU/ml)	$\geq10^4$	No defined	2.59×10^6	2.42×10^6	12.00	meet the D-2 standard and Guideline 8
	Escherichia coli(CFU/100ml)	>2500	<250	30.67×10^2	16.00×10^2	0.67	meet the D-2 standard and Guideline 8
	Intestinal Enterococci(CFU/100ml)	>1000	<100	16.00×10^2	11.30×10^2	0.56	meet the D-2 standard and Guideline 8
	Vibrio choleerae(CFU/100ml)	>10	<1	6.33×10^2	6.70×10^2	0	meet the D-2 standard and Guideline 8
Trial 3	≥50 μm (ind/m^3)	>100	<10	3.76×10^2	2.87×10^3	no living organism	meet the D-2 standard and Guideline 8
	10 μm ~50 μm (cell/ml)	>100	<10	4.03×10^2	1.19×10^2	no living organism	meet the D-2 standard and Guideline 8
	<10μm -Bacteria(CFU/100ml)	$\geq10^4$	No defined	1.37×10^6	1.19×10^6	6.22	meet the D-2 standard and Guideline 8
	Escherichia coli(CFU/100ml)	>2500	<250	25.00×10^2	16.3×10^2	0.33	meet the D-2 standard and Guideline 8
	Intestinal Enterococci(CFU/100ml)	>1000	<100	10.33×10^2	7.00×10^2	0.22	meet the D-2 standard and Guideline 8
	Vibrio choleerae(CFU/100ml)	>10	<1	2.30×10^2	1.60×10^2	0	meet the D-2 standard and Guideline 8
Trial 4	≥50 μm (ind/m^3)	>100	<10	1.61×10^4	5.60×10^2	no living organism	meet the D-2 standard and Guideline 8
	10 μm ~50 μm (cell/ml)	>100	<10	4.50×10^4	1.38×10^3	no living organism	meet the D-2 standard and Guideline 8
	<10μm Bacteria(CFU/100ml)	$\geq10^4$	No defined	1.33×10^5	1.99×10^5	0	meet the D-2 standard and Guideline 8
	Escherichia coli(CFU/100ml)	>2500	<250	3.90×10^3	4.46×10^3	0	meet the D-2 standard and Guideline 8
	Intestinal Enterococci(CFU/100ml)	>1000	<100	10.70×10^2	8.70×10^2	0	meet the D-2 standard and Guideline 8
	Vibrio choleerae(CFU/100ml)	>10	<1	7.70×10^2	9.27×10^2	0	meet the D-2 standard and Guideline 8

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Appendix 1 **Results for water quality parameters of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycles of trials	Number	Temperature(℃)	Salinity	pH	NTU	TSS(mg/L)	DO（mg/L）	DOC(mg/L)	POC(mg/L)
2010.7.19	Qingdao	Influent ballasting water of reference tank in cycle 1	SST1-1B1/d	23.8	31.2	7.92	2.28	21.80	7.25	5.69	0.49
			SST1-1M1/d	23.7	31.6	7.88	2.63	29.80	7.10	1.43	0.61
			SST1-1E1/d	23.6	31.3	7.89	2.28	31.20	7.35	3.52	0.40
2010.7.24	Dongguan	Effluent de-ballasting water of treated tank in cycle 1	SST2-1B1/d	29.2	31.3	7.92	3.07	9.50	6.54	1.58	0.21
			SST2-1B2/d			7.97	3.33	11.88	6.48	1.43	0.31
			SST2-1B3/d			7.95	3.03	11.25	6.39	1.37	0.34
			SST2-1M1/d			7.93	3.16	7.20	6.15	1.86	0.30
			SST2-1M2/d			7.94	2.98	9.70	6.30	2.15	0.20
			SST2-1M3/d			7.93	3.38	12.13	6.49	1.68	0.18
			SST2-1E1/d			7.93	3.42	10.75	6.42	2.48	0.24
			SST2-1E2/d			7.95	2.89	11.63	6.59	2.12	0.13
			SST2-1E3/d	29.3	30.7	7.95	3.25	10.50	6.10	2.31	0.16
2010.7.24	Dongguan	Effluent de-ballasting water of reference tank in cycle 1	SST3-1B1/d			7.95	3.95	12.00	6.91	1.84	0.16
			SST3-1M1/d			8.00	5.04	13.50	6.64	2.13	0.14
			SST3-1E1/d			7.98	5.22	31.64	6.59	1.63	0.15

Analyst 潘婷萍 Proofreader 王保林

Appendix 1 **Results for water quality parameters of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycles of trials	Number	Temperature(℃)	Salinity	pH	NTU	TSS(mg/L)	DO（mg/L）	DOC(mg/L)	POC(mg/L)
2010.7.24	Dongguan	Influent ballasting water of reference tank in cycle 2	SST1-2B1/d	29.9	0.8	7.70	29.52	50.50	4.29	1.92	2.09
			SST1-2M1/d			7.69	25.31	35.50	3.98	1.72	2.20
			SST1-2E1/d			7.67	24.04	40.00	4.31	3.20	2.03
2010.7.29	Qingdao	Effluent de-ballasting water of treated tank in cycle 2	SST2-2B1/d	25.8	1.3	7.23	2.24	2.00	4.15	2.18	0.36
			SST2-2B2/d			7.11	2.02	2.67	4.29	2.01	0.31
			SST2-2B3/d			7.13	2.06	7.67	4.37	2.24	0.34
			SST2-2M1/d			7.10	1.80	4.33	4.12	2.34	0.35
			SST2-2M2/d			7.17	1.71	5.00	4.46	2.26	0.34
			SST2-2M3/d			7.16	1.62	6.67	4.28	2.43	0.31
			SST2-2E1/d			7.18	1.84	8.33	4.46	2.34	0.36
			SST2-2E2/d			7.19	2.28	9.00	4.53	2.25	0.34
			SST2-2E3/d			7.20	2.46	8.67	4.43	2.31	0.37
2010.7.29	Qingdao	Effluent de-ballasting water of reference tank in cycle 2	SST3-2B1/d			7.00	1.75	7.33	4.62	2.15	0.41
			SST3-2M1/d			7.02	1.62	11.67	5.68	1.61	0.38
			SST3-2E1/d			7.05	1.93	7.00	5.02	1.83	0.43

Analyst 谢[illegible] Proofreader [illegible]

Appendix 1

Results for water quality parameters of the shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycles of trials	Number	Temperature(℃)	Salinity	pH	NTU	TSS(mg/L)	DO（mg/L）	DOC(mg/L)	POC(mg/L)
2010.8.10	Zhoushan	Influent ballasting water of reference tank in cycle 3	SST1-3B1/d	25.8	27.8	7.92	13.64	51.50	6.31	1.29	0.36
			SST1-3M1/d	25.9	27.8	7.93	16.67	52.25	6.49	1.75	0.37
			SST1-3E1/d	25.8	27.8	7.93	11.93	37.75	6.28	1.15	0.30
2010.8.15	Qingdao	Effluent de-ballasting water of treated tank in cycle 3	SST2-3B1/d	26.2	27.6	7.89	0.57	8.25	6.46	1.54	0.13
			SST2-3B2/d			7.87	0.31	8.88	6.29	1.40	0.11
			SST2-3B3/d			7.88	0.31	10.13	6.25	9.63	0.10
			SST2-3M1/d			7.92	0.39	9.25	6.25	1.38	0.09
			SST2-3M2/d			7.91	0.39	8.00	6.40	1.51	0.11
			SST2-3M3/d			7.91	0.44	9.25	6.31	1.56	0.17
			SST2-3E1/d			7.92	0.57	10.75	6.49	4.00	0.12
			SST2-3E2/d			7.92	0.57	9.88	6.46	1.35	0.11
			SST2-3E3/d			7.94	0.96	10.63	6.55	2.79	0.11
2010.8.15	Qingdao	Effluent de-ballasting water of reference tank in cycle 3	SST3-3B1/d			7.83	0.57	7.25	6.48	2.23	0.13
			SST3-3M1/d			7.89	0.88	10.13	6.39	1.43	0.12
			SST3-3E1/d			7.91	1.40	10.38	6.34	1.32	0.13

Analyst 潘琳琳 Proofreader 王保林

Appendix 1 **Results for water quality parameters of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycles of trials	Number	Temperature(℃)	Salinity	pH	NTU	TSS(mg/L)	DO（mg/L）	DOC(mg/L)	POC(mg/L)
2011.1.21	Dongguan	Influent ballasting water of reference tank in cycle 4	SST1-4B1/d	6.4	15.4	7.36	10.22	52.70	6.31	4.05	0.35
			SST1-4M1/d	6.8	15.6	7.36	10.11	36.20	6.24	3.38	0.26
			SST1-4E1/d	6.9	15.2	7.34	9.49	54.20	6.33	4.29	0.37
2011.1.28	Qingdao	Effluent de-ballasting water of treated tank in cycle 4	SST2-4B1/d	5.7	4.3	7.71	1.37	1.77	6.46	2.59	0.15
			SST2-4B2/d	6.0	4.3	7.71	2.07	1.77	6.37	2.87	0.13
			SST2-4B3/d	6.1	4.4	7.58	1.46	3.63	6.49	3.03	0.18
			SST2-4M1/d	5.1	4.3	7.72	2.11	2.06	6.97	2.72	0.21
			SST2-4M2/d	5.7	4.1	7.61	1.86	1.63	6.80	2.82	0.20
			SST2-4M3/d	6.0	4.1	7.58	2.12	5.58	6.56	3.11	0.15
			SST2-4E1/d	6.1	4.0	7.73	8.51	31.06	5.68	2.80	0.46
			SST2-4E2/d	6.0	4.0	7.72	9.29	42.87	6.23	2.73	0.55
			SST2-4E3/d	6.1	4.0	7.72	9.47	50.40	6.39	2.84	0.56
2011.1.28	Qingdao	Effluent de-ballasting water of reference tank in cycle 4	SST3-4B1/d	8.0	4.2	7.61	1.51	1.77	6.33	2.58	0.23
			SST3-4B2/d	7.9	4.1	7.54	1.50	3.34	6.22	2.53	0.22
			SST3-4B3/d	7.9	4.0	7.61	1.48	4.57	6.42	2.37	0.14

Analyst 潘打打捞 Proofreader 王保栋

Appendix 2

Results for organisms (≥50 μm) of shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of filtering (m^3)	Sampling proportion	(Dominant Species)	Density of viable organisms cell·ml^{-1}	Total Density cell·ml^{-1}	Density of death cell·ml^{-1}
2010.7.19	Qingdao	Influent ballasting water of reference tank in cycle 1	SST1-1B1/a	1	1/40	Cyclopoida	27440	58922	
						Acartia pacifica	9240		
						late Nauplius larvae	7520		
						Lamellibranchia larvae	5240		
						Paracalanus parvus	3760		
						Cirripedia nauplius	3160		
						Copepoda larvea	1080		
			SST1-1M1/a	1	1/40	Cyclopoida	35640	58040	
						Acartia pacifica	8680		
						late Nauplius larvae	6480		
						Lamellibranchia larvae	2440		
						Copepoda larvea	1880		
						Paracalanus parvus	1080		
						Cirripedia nauplius	760		
			SST1-1E1/a	1	1/40	Cyclopoida	11540	21068	
						Acartia pacifica	5160		
						late Nauplius larvae	1840		
						Paracalanus parvus	1340		
						Cirripedia nauplius	520		
2010.7.24	Dongguan	Effluent de-ballasting water of treated tank in cycle 1	SST2-1B1/a	1	100%	no viable organisms observed	0	0	
			SST2-1B2/a	1	100%	no viable organisms observed	0	0	
			SST2-1B3/a	1	100%	no viable organisms observed	0	0	
			SST2-1M1/a	1	100%	no viable organisms observed	0	0	
			SST2-1M2/a	1	100%	no viable organisms observed	0	0	
			SST2-1M3/a	1	100%	no viable organisms observed	0	0	
			SST2-1E1/a	1	100%	no viable organisms observed	0	0	
			SST2-1E2/a	1	100%	no viable organisms observed	0	0	
			SST2-1E3/a	1	100%	no viable organisms observed	0	0	
		Effluent de-ballasting water of reference tank in cycle 1	SST3-1B1/a	1	100%	*Acartia pacifica*	29	85	
						Corycaeus affinis	19		
						Ostracoda	11		
						Cyclopoida	8		
			SST3-1M1/a	1	100%	*Acartia pacifica*	45	204	
						Lamellibranchia larvae	39		
						Cyclopoida	36		
						Ostracoda	29		
						Corycaeus affinis	27		
			SST3-1E1/a	1	100%	*Corycaeus affinis*	41	128	
						Ostracoda	28		
						Lamellibranchia larvae	21		
						Acartia pacifica	19		
						Cyclopoida	14		

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Appendix 2 Results for organisms (≥50 μm) of shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of filtering (m^3)	Sampling proportion	(Dominant Species)	Density of viable organisms cell·ml^{-1}	Total Density cell·ml^{-1}	Density of death cell·ml^{-1}
2010.7.24	Dongguan	Influent ballasting water of reference tank in cycle 2	SST1-2B1/a	1	1/10	Cyclopoida	640	1693	
						Sagitta crassa	372		
						Ostracoda	330		
						late Nauplius larvae	180		
						Calanus sinicus	100		
			SST1-2M1/a	1	1/20	Cyclopoida	1140	2340	
						late Nauplius larvae	960		
						Ostracoda	160		
						Polychaeta larvae	80		
			SST1-2E1/a	1	1/20	late Nauplius larvae	9640	16101	
						Cyclopoida	5860		
						Ostracoda	600		
						Caprella sp.	1		
2010.7.29	Qingdao	Effluent de-ballasting water of treated tank in cycle 2	SST2-2B1/a	1	100%	Cyclopoida	0	14	13
						Ostracoda	0		1
			SST2-2B2/a	1	100%	Cyclopoida	0	25	25
			SST2-2B3/a	1	100%	Cyclopoida	0	30	30
			SST2-2M1/a	1	100%	Cyclopoida	0	46	45
						Corycaeus affinis	0		1
			SST2-2M2/a	1	100%	Cyclopoida	0	70	70
			SST2-2M3/a	1	100%	Cyclopoida	0	57	57
			SST2-2E1/a	1	100%	Cyclopoida	0	78	77
						Polychaeta larvae	0		1
			SST2-2E2/a	1	100%	Cyclopoida	0	29	29
			SST2-2E3/a	1	100%	Cyclopoida	0	108	107
		Effluent de-ballasting water of reference tank in cycle 2	SST3-2B1/a	1	1/20	Cyclopoida	1440	3502	
						Calanus sinicus	860		
						late Nauplius larvae	480		
						Sagitta crassa	337		
						Corycaeus affinis	220		
			SST3-2M1/a	1	1/20	Cyclopoida	2080	3040	
						late Nauplius larvae	940		
						Polychaeta larvae	20		
			SST3-2E1/a	1	1/20	Cyclopoida	2520	3975	
						late Nauplius larvae	800		
						Calanus sinicus	480		

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Appendix 2

Results for organisms (≥50 μm) of shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of filtering (m^3)	Sampling proportion	(Dominant Species)	Density of viable organisms cell·ml^{-1}	Total Density cell·ml^{-1}	Density of death cell·ml^{-1}
2010.8.10	Zhoushan	Influent ballasting water of reference tank in cycle 3	SST1-3B1/a	1	1/20	*Paracalanus parvus*	1420	3434	
						Copepoda larvea	420		
						Sagitta spp.	389		
						late Nauplius larvae	320		
						Harpacticoida sp.	200		
						Calyptopis larvae	200		
			SST1-3M1/a	1	1/20	late Nauplius larvae	1340	3935	
						Paracalanus parvus	880		
						Harpacticoida sp.	600		
						Corycaeus affinis	480		
						Polychaeta larvae	240		
			SST1-3E1/a	1	1/20	*Corycaeus affinis*	1180	3933	
						Calanus sinicus	660		
						late Nauplius larvae	500		
						Harpacticoida sp.	360		
						Calyptopis larvae	300		
						Paracalanus parvus	200		
2010.8.15	Qingdao	Effluent de-ballasting water of treated tank in cycle 3	SST2-3B1/a	1	100%	Ostracoda	0	19	1
						Centropages dorsispinatus	0		7
						Corycaeus affinis	0		5
						Cyclopoida	0		4
						Harpacticoida sp.	0		1
						Cirripedia nauplius	0		1
			SST2-3B2/a	1	100%	*Harpacticoida* sp.	0	46	12
						Copepoda larvea	0		12
						Cyclopoida	0		12
						Corycaeus affinis	0		6
						Centropages dorsispinatus	0		4
			SST2-3B3/a	1	100%	Ostracoda	0	27	1
						Harpacticoida sp.	0		0
						Cyclopoida	0		19
						Corycaeus affinis	0		5
						late Nauplius larvae	0		2
			SST2-3M1/a	1	100%	no viable organisms observed	0	0	
			SST2-3M2/a	1	100%	no viable organisms observed	0	0	
			SST2-3M3/a	1	100%	no viable organisms observed	0	0	
			SST2-3E1/a	1	100%	no viable organisms observed	0	0	
			SST2-3E2/a	1	100%	no viable organisms observed	0	0	
			SST2-3E3/a	1	100%	no viable organisms observed	0	0	
		Effluent de-ballasting water of reference tank in cycle 3	SST3-3B1/a	1	1/10	late Nauplius larvae	670	1543	
						Copepoda larvea	330		
						Harpacticoida sp.	250		
						Corycaeus affinis	190		
			SST3-3M1/a	1	1/20	late Nauplius larvae	1740	3600	
						Harpacticoida sp.	800		
						Corycaeus affinis	560		
						Copepoda larvea	380		
			SST3-3E1/a	1	1/20	late Nauplius larvae	1800	3460	
						Copepoda larvea	640		
						Harpacticoida sp.	540		
						Corycaeus affinis	300		

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Appendix 2

Results for organisms (≥50 μm) of shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of filtering (m^3)	Sampling proportion	(Dominant Species)	Density of viable organisms cell·ml^{-1}	Total Density cell·ml^{-1}	Density of death cell·ml^{-1}
2011.1.21	Dongguan	Influent ballasting water of reference tank in cycle 4	SST1-4B1/a	1	1/40	*Schmackeria* sp.	15080	16240	
						Cyclopoida	520		
						late Nauplius larvae	600		
						Sinocalanus sp.	40		
			SST1-4M1/a	1	1/40	*Schmackeria* sp.	15840	17440	
						Cyclopoida	640		
						late Nauplius larvae	840		
						Sinocalanus sp.	80		
						Acartia sp.	40		
			SST1-4E1/a	1	1/40	*Schmackeria* sp.	13920	14840	
						Cyclopoida	440		
						late Nauplius larvae	480		
2011.1.28	Qingdao	Effluent de-ballasting water of treated tank in cycle 4	SST2-4B1/a	1	100%	*Schmackeria* sp.	0	1	1
			SST2-4B2/a	1	100%	*Schmackeria* sp.	0	489	47
						Cyclopoida	0		423
						late Nauplius larvae	0		17
						Acanthomysis sp.	0		2
			SST2-4B3/a	1	100%	no viable organisms observed	0	0	
			SST2-4M1/a	1	100%	no viable organisms observed	0	0	
			SST2-4M2/a	1	100%	no viable organisms observed	0	0	
			SST2-4M3/a	1	100%	no viable organisms observed	0	0	
			SST2-4E1/a	1	100%	no viable organisms observed	0	0	
			SST2-4E2/a	1	100%	no viable organisms observed	0	0	
			SST2-4E3/a	1	100%	no viable organisms observed	0	0	
		Effluent de-ballasting water of reference tank in cycle 4	SST3-4B1/a	1	1/20	*Schmackeria* sp.	180	260	
						Cyclopoida	40		
						late Nauplius larvae	40		
			SST3-4M1/a	1	1/20	*Schmackeria* sp.	280	560	
						Cyclopoida	140		
						late Nauplius larvae	120		
			SST3-4E1/a	1	1/20	*Schmackeria sp.*	200	861	
						Cyclopoida	280		
						late Nauplius larvae	360		

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Appendix 3 **Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2010.7.19	Qingdao	Influent ballasting water of reference tank in cycle 1	SST1-1B1/b	1	15	Dinophyta	*Ceratium lineatum*	112.5	193.5
							Dinophysin acuminata	13.5	
							Ceratium tripos	4.5	
						Bacillariophyta	*Coscinodiscus* sp.	9	
							Coscinodiscus waileii	9	
							Chaetoceros curvisetus	31.5	
							Actinoptychus sp.	4.5	
						Chrysophyta	*Dictocha fibula*	9	
			SST1-1E1/b	1	15	Dinophyta	*Ceratium lineatum*	130.5	216
							Ceratium fusus	18	
							Dinophysin acuminata	9	
							Dinophysis fortii	4.5	
							Gyrodinium spirace	4.5	
						Bacillariophyta	*Actinoptychus* sp.	4.5	
							Chaetoceros curvisetus	31.5	
						Chrysophyta	*Dictocha fibula*	13.5	
			SST1-1M1/b	1	15	Dinophyta	*Ceratium lineatum*	139.5	333
							Dinophysin acuminata	18	
							Ceratium fusus	13.5	
							Alexandrium sp.	4.5	
							Ceratium horridum	4.5	
							Noctiluca scintillans	4.5	
						Bacillariophyta	*Coscinodiscus oculus-iridio*	31.5	
							Actinoptychus sp.	31.5	
							Skeletonema costatum	27	
							Nitzschia closterium	18	
							Coscinodiscus sp.	13.5	
							Coscinodiscus asteromphalus	4.5	
							Ditycum brightuellii	4.5	
							Streptotheca thamesis	4.5	
							Coscinodiscus waileii	4.5	
						Chrysophyta	*Dictocha fibula*	9	

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Appendix 3 Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2010.7.24	Dongguan	Effluent de-ballasting water of treated tank in cycle 1	SST2-1B1/b	1	10	no viable organisms observed		0	0
			SST2-1B2/b	1	10	no viable organisms observed		0	0
			SST2-1B3/b	1	10	no viable organisms observed		0	0
			SST2-1M1/b	1	10	no viable organisms observed		0	0
			SST2-1M2/b	1	10	no viable organisms observed		0	0
			SST2-1M3/b	1	10	no viable organisms observed		0	0
			SST2-1E1/b	1	10	no viable organisms observed		0	0
			SST2-1E2/b	1	10	no viable organisms observed		0	0
			SST2-1E3/b	1	10	no viable organisms observed		0	0
2010.7.24	Dongguan	Effluent de-ballasting water of reference tank in cycle 1	SST3-1E1/b	1	10	Bacillariophyta	*Leptocylindrus mediterraneus*	92	161.2
							Skeletonema costatum	12	
							Coscinodiscus sp.	7.2	
							Leptocylindrus danicus	6	
						Dinophyta	*Ceratium lineatum*	42	
							Ceratium fusus	2	
			SST3-1M1/b	1	15	Dinophyta	*Ceratium lineatum*	31.5	63
						Bacillariophyta	*Coscinodiscus* sp.	18	
							Coscinodiscus gigas	4.5	
						Chrysophyta	*Dictocha fibula*	9	
			SST3-1B1/b	1	10	Bacillariophyta	*Leptocylindrus mediterraneus*	26	72
							Rhizosolenia delicatula	6	
							Coscinodiscus sp.	2	
						Dinophyta	*Ceratium lineatum*	26	
							Dinophysin acuminata	4	
							Akashiwo sanguiniea	2	
							Protoperidinium steinii	2	
							Ceratium tripos	2	
							Ceratium fusus	2	

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Appendix 3 **Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2010.7.24	Dongguan	Influent ballasting water of reference tank in cycle 2	SST1-2B1/b	1	10	Bacillariophyta	*Melosira granulata*	1700	2713.3
							Cyclotella sp.	299.2	
							Synedra sp.	4.8	
						Chlorophyta	*Actinastrum hantschii*	320.0	
							Pediastrum duplex	96.4	
							Cosmarium sp.	30.0	
							Scenedesums quadricauda	8.0	
							Scanedesums denticulatus	1.9	
							Tetrastrum hastiferum	1.6	
							Staurastrum zahlbruckneri	0.4	
							Tetrastrum heterocanthum	0.4	
						Euglenophyta	*Euglena* spp.	5.2	
							Trachelomonas sp.	1.0	
						Dinophyta	*Gymnodinium* sp.	2.8	
						others	Others	241.6	
			SST1-2M1/b	1	20	Bacillariophyta	*Melosira granulata*	2548.80	9323.2
							Cyclotella sp.	227.20	
							Synedra sp.	40.00	
							Skeletonema costatum	14.40	
						Chlorophyta	*Actinastrum hantschii*	5328.00	
							Pediastrum duplex	635.20	
							Scenedesums dimorphus	307.20	
						Euglenophyta	*Euglena* spp.	1.60	
						others	Others	220.80	
			SST1-2E1/b	1	20	Bacillariophyta	*Melosira granulata*	2627.20	3865.76
							Cyclotella sp.	140.80	
							Synedra sp.	30.40	
							Cocconeis sp.	0.08	
						Chlorophyta	*Pediastrum duplex*	332.80	
							Actinastrum hantschii	190.40	
							Scenedesums dimorphus	187.20	
							Scenedesums quadricauda	161.60	
							Ankistrodesmus acicularis	27.20	
							Scenedesums sp.	0.08	
						Cyanophyta	*Spirutina platensis*	147.20	
						Euglenophyta	*Euglena* spp.	20.80	

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Appendix 3 **Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2010.7.29	Qingdao	Effluent de-ballasting water of treated tank in cycle 2	SST2-2B1/b	1	55	no viable organisms observed			0.33
			SST2-2B2/b	1	55	no viable organisms observed			
			SST2-2B3/b	1	51	no viable organisms observed			
			SST2-2M1/b	1	63	Bacillariophyta	*Melosira granulata*	0.07	
			SST2-2M2/b	1	69	no viable organisms observed			
			SST2-2M3/b	1	57	no viable organisms observed			
			SST2-2E1/b	1	59	no viable organisms observed			
			SST2-2E2/b	1	47	Bacillariophyta	*Melosira granulata*	0.26	
			SST2-2E3/b	1	91	no viable organisms observed			
2010.7.29	Qingdao	Effluent de-ballasting water of reference tank in cycle 2	SST3-2B1/b	1	63	Bacillariophyta	*Melosira granulata*	405.62	919.91
							Cyclotella sp.	116.24	
							Nitzschia sp.	4.31	
							Coscinodiscus sp.	1.47	
						Chlorophyta	*Pediastrum boryanum*	55.34	
							Scenedesums sp.	32.76	
							Actinastrum hantschii	29.30	
						Cyanophyta	*Trichodesmium* sp.	168.00	
							Spiralina sp.	3.89	
						others	Others	103.01	
			SST3-2M1/b	1	80	Bacillariophyta	*Melosira granulata*	514.26	1583.73
							Cyclotella sp.	225.47	
							Nitzschia sp.	12.27	
							Coscinodiscus sp.	0.53	
						Chlorophyta	*Pediastrum boryanum*	62.40	
							Scenedesums sp.	81.87	
							Actinastrum hantschii	84.27	
						Cyanophyta	*Trichodesmium* sp.	486.67	
							Spiralina sp.	6.67	
						others	Others	109.33	
			SST3-2E1/b	1	15	Bacillariophyta	*Melosira granulata*	107.38	281.53
							Cyclotella sp.	40.23	
							Nitzschia sp.	3.33	
							Coscinodiscus sp.	1.83	
						Cyanophyta	*Trichodesmium* sp.	95.00	
							Spiralina sp.	0.93	
						Chlorophyta	*Scenedesums* sp.	10.88	
							Actinastrum hantschii	8.93	
							Pediastrum boryanum	4.58	
						others	Others	8.48	

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Appendix 3 **Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2010.8.10	Zhoushan	Influent ballasting water of reference tank in cycle 3	SST1-3B1/b	1	10	Bacillariophyta	*Skeletonema costatum*	90	409.3
							Pseudonitzschia pungens	66	
							Rhizosolenia setigera	50	
							Melosira sulcata	44	
							Coscinodiscus radiatus	34	
							Chaetoceros sp.	28	
							Chaetoceros compressus	16	
							Chaetoceros curvisetus	16	
							Cyclotella sp.	16	
							Chaetoceros lorenzianus	15.3	
							Nitzschia closterium	8	
							Coscinodiscus jonesianus	4	
							Rhizosolenia alata f. gracillima	4	
							Coscinodiscus asteromphalus	2	
							Ditylum brightwellii	2	
						Dinophyta	*Ceratium fusus*	6	
							Ceratium furca	4	
							Ceratium tripos	4	
			SST1-3M1/b	1	10	Bacillariophyta	*Skeletonema costatum*	128	548
							Chaetoceros lorenzianus	122	
							Coscinodiscus jonesianus	58	
							Chaetoceros curvisetus	36	
							Rhizosolenia setigera	22	
							Chaetoceros compressus	22	
							Pseudonitzschia pungens	20	
							Coscinodiscus radiatus	16	
							Melosira sulcata	12	
							Actinoptychus sp.	8	
							Rhizosolenia alata f. *gracillima*	8	
							Ditylum brightwellii	6	
							Gyrosigma sp.	4	
							Podocystis sp.	4	
							Thalassiothrix frauenfeldii	4	
							Chaetoceros sp.	4	
							Coscinodiscus asteromphalus	4	
							Chaetoceros castracanei	2	
							Coscinodiscus excentricus	2	
							Pleurosigma pelagicum	2	
							Pleurosigma sp.	2	
							Nitzschia closterium	2	
						Dinophyta	*Ceratium tripos*	22	
							Ceratium furca	18	
							Ceratium fusus	12	
							Protoperidinium sp.	6	
							Protoperidinium pallidum	2	

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Appendix 3 Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2010.8.10	Zhoushan	Influent ballasting water of reference tank in cycle 3	SST1-3E1/b	1	10	Bacillariophyta	*Skeletonema costatum*	62	254
							Chaetoceros lorenzianus	58	
							Pseudonitzschia pungens	46	
							Rhizosolenia setigera	30	
							Actinoptychus sp.	8	
							Leptocylindrus danicus	6	
							Chaetoceros compressus	4	
							Rhizosolenia alata f. *gracillima*	4	
							Corethron hystrix	2	
							Coscinodiscus radiatus	2	
							Thalassiothrix frauenfeldii	2	
						Dinophyta	*Ceratium furca*	12	
							Ceratium fusus	10	
							Alexandrium sp.	4	
							Dinoflagellates	2	
							Protoperidinium pentagonum	2	
2010.8.15	Qingdao	Effluent de-ballasting water of treated tank in cycle 3	SST2-3B1/b	1	10	no viable organisms observed		0	0
			SST2-3B2/b	1	10	no viable organisms observed		0	0
			SST2-3B3/b	1	10	no viable organisms observed		0	0
			SST2-3M1/b	1	10	no viable organisms observed		0	0
			SST2-3M2/b	1	10	no viable organisms observed		0	0
			SST2-3M3/b	1	10	no viable organisms observed		0	0
			SST2-3E1/b	1	10	no viable organisms observed		0	0
			SST2-3E2/b	1	10	no viable organisms observed		0	0
			SST2-3E3/b	1	10	no viable organisms observed		0	0

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Appendix 3 Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration(ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2010.8.15	Qingdao	Effluent de-ballasting water of reference tank in cycle 3	SST3-3B1/b	1	10	Bacillariophyta	*Skeletonema costatum*	40	82
							Chaetoceros curvisetus	6	
							Chaetoceros lorenzianus	6	
							Chaetoceros affinis	4	
							Pseudonitzschia pungens	4	
							Eucampia zodiacus	2	
							Ditylum brightwellii	2	
							Nitzschia closterium	2	
						Dinophyta	*Ceratium fusus*	8	
							Ceratium tripos	4	
							Protoperidinium sp.	4	
			SST3-3M1/b	1	10	Bacillariophyta	*Skeletonema costatum*	66	132
							Pseudonitzschia pungens	12	
							Chaetoceros lorenzianus	10	
							Chaetoceros sp.	6	
							Rhizosolenia setigera	6	
							Rhizosolenia styliformis	2	
							Rhiz.alata f. gracillima	2	
							Gyrosigma sp.	2	
							Ditylum brightwellii	2	
						Dinophyta	*Protoperidinium* sp.	10	
							Ceratium fusus	8	
							Ceratium tripos	4	
							Ceratium furca	2	
			SST3-3E1/b	1	10	Bacillariophyta	*Chaetoceros lorenzianus*	62	142
							Chaetoceros curvisetus	12	
							Skeletonema costatum	12	
							Pseudonitzschia pungens	12	
							Rhizosolenia setigera	10	
							Thalassionema nitzschioides	6	
							Chaetoceros compressus	6	
							Coscinodiscus gigas	2	
							Eucampia zodiacus	2	
						Dinophyta	*Ceratium fusus*	6	
							Ceratium furca	6	
							Alexandrium sp.	2	
							Protoperidinium sp.	2	
							Ceratium tripos	2	

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Appendix 3 **Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2011.1.21	Dongguan	Influent ballasting water of reference tank in cycle 4	SST1-4B1/b	1	20	Bacillariophyta	*Coscinodiscus* spp.	1890	5301
							Paralia sulcata	645	
							Thalassiosira spp.	415	
						Chlorophyta	*Microspora stagnorum*	968	
							Hormidium spp.	738	
							Scenedesmus quadricauda	184	
							Scenedesmus carinatus	184	
							Scenedesmus dimorphus	184	
							Pediastrum spp.	92	
			SST1-4M1/b	1	20	Bacillariophyta	*Paralia sulcata*	760	3940
							Thalassiosira spp.	467	
							Coscinodiscus spp.	415	
							Leptocylindrus spp.	242	
							Nitzschia spp.	17	
						Chlorophyta	*Hormidium* spp.	934	
							Pediastrum biradiatum	397	
							Pediastrum duplex	276	
							Palmella mucosa	138	
							Scenedesmus dimorphus	138	
							Scenedesmus quadricauda	69	
							Scenedesmus carinatus	69	
							Pediastrum spp.	17	
			SST1-4E1/b	1	20	Bacillariophyta	*Thalassiosira* spp.	1088	4239
							Coscinodiscus spp.	843	
							Paralia sulcata	466	
							Leptocylindrus spp.	111	
							Nitzschia spp.	44	
						Chlorophyta	*Microspora stagnorum*	1065	
							Scenedesmus armatus	200	
							Scenedesmus dimorphus	178	
							Scenedesmus javaensis	155	
							Scenedesmus carinatus	89	

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Appendix 3 Results for organisms (10 μm-50 μm) of the shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycle	Number	Volume of samples (L)	Volume of contration (ml)	(Dominant Species)		(Density) cell·ml^{-1}	(Total Density) cell·ml^{-1}
2011.1.28	Qingdao	Effluent de-ballasting water of treated tank in cycle 4	SST2-4B1/b	1	10	no viable organisms observed		0	0
			SST2-4B2/b	1	10	no viable organisms observed		0	0
			SST2-4B3/b	1	10	no viable organisms observed		0	0
			SST2-4M1/b	1	10	no viable organisms observed		0	0
			SST2-4M2/b	1	10	no viable organisms observed		0	0
			SST2-4M3/b	1	10	no viable organisms observed		0	0
			SST2-4E1/b	1	10	no viable organisms observed		0	0
			SST2-4E2/b	1	10	no viable organisms observed		0	0
			SST2-4E3/b	1	10	no viable organisms observed		0	0
2011.1.28	Qingdao	Effluent de-ballasting water of reference tank in cycle 4	SST3-24B1/b	1	15	protozoa	Protozoa	1659	2834
						Bacillariophyta	*Paralia sulcata*	622	
							Thalassiosira spp.	346	
							Coscinodiscus spp.	207	
			SST3-4M1/b	1	15	protozoa	Protozoa	246	553
						Bacillariophyta	*Paralia sulcata*	154	
							Thalassiosira spp.	123	
							Coscinodiscus spp.	31	
			SST3-4E1/b	1	15	protozoa	Protozoa	650	772
						Bacillariophyta	*Thalassiosira* spp.	81	
							Nitzschia spp.	41	

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Appendix 4 **Results for microbes of shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycles of trials	Number	H.bacteria（CFU/100mL）	V.cholerae（CFU/100ml）	E.coli（CFU/100ml）	I.enterococci（CFU/100ml）
2010.7.19	Qindao	Influent ballasting water of reference tank in cycle 1	SST1-1B1/C	3.50×10^6	5×10^2	20×10^2	34×10^2
			SST1-1M1/C	2.78×10^6	10×10^2	52×10^2	5×10^2
			SST1-1E1/C	3.12×10^6	2×10^2	8×10^2	7×10^2
2010.7.24	Dongguan	Effluent de-ballasting water of treated tank in cycle 1	SST2-1B1/C	0	0	1	3
			SST2-1B2/C	8	0	0	0
			SST2-1B3/C	0	0	1	0
			SST2-1M1/C	0	0	0	0
			SST2-1M2/C	0	0	0	0
			SST2-1M3/C	15	0	3	1
			SST2-1E1/C	0	0	0	0
			SST2-1E2/C	3	0	0	0
			SST2-1E3/C	0	0	0	0
2010.7.24	Dongguan	Effluent de-ballasting water of reference tank in cycle 1	SST3-1B1/C	1.1×10^6	4×10^2	5×10^2	4×10^2
			SST3-1M1/C	2.3×10^6	14×10^2	28×10^2	8×10^2
			SST3-1E1/C	2.9×10^6	4×10^2	8×10^2	3×10^2

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Appendix 4 **Results for microbes of shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycles of trials	Number	H.bacteria（CFU/100mL）	V.cholerae（CFU/100ml）	E.coli（CFU/100ml）	I.enterococci（CFU/100ml）
2010.7.24	Dongguan	Influent ballasting water of reference tank in cycle 2	SST1-2B1/C	8.7×10^5	7×10^2	24×10^2	28×10^2
			SST1-2M1/C	2.59×10^6	4×10^2	56×10^2	9×10^2
			SST1-2E1/C	4.3×10^6	8×10^2	12×10^2	11×10^2
2010.7.29	Qindao	Effluent de-ballasting water of treated tank in cycle 2	SST2-2B1/C	1	0	0	1
			SST2-2B2/C	0	0	0	2
			SST2-2B3/C	44	0	3	0
			SST2-2M1/C	31	0	0	0
			SST2-2M2/C	0	0	0	0
			SST2-2M3/C	15	0	2	1
			SST2-2E1/C	12	0	1	0
			SST2-2E2/C	5	0	0	1
			SST2-2E3/C	0	0	0	0
2010.7.29	Qindao	Effluent de-ballasting water of reference tank in cycle 2	SST3-2B1/C	0.26×10^6	4×10^2	17×10^2	13×10^2
			SST3-2M1/C	2.4×10^6	5×10^2	22×10^2	7×10^2
			SST3-2E1/C	4.6×10^6	11×10^2	9×10^2	14×10^2

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Appendix 4

Results for microbes of shipboard trails of *BSKY*™

Sampling date	Site of trials	Cycles of trials	Number	H.bacteria（CFU/100mL）	V.cholerae（CFU/100ml）	E.coli（CFU/100ml）	I.enterococci（CFU/100ml）
2010.8.10	Zhoushan	Influent ballasting water of reference tank in cycle 3	SST1-3B1/C	7.2×10^5	1×10^2	24×10^2	21×10^2
			SST1-3M1/C	2.1×10^6	4×10^2	17×10^2	7×10^2
			SST1-3E1/C	1.3×10^6	2×10^2	22×10^2	3×10^2
2010.8.15	Qindao	Effluent de-ballasting water of treated tank in cycle 3	SST2-3B1/C	4	0	1	2
			SST2-3B2/C	3	0	0	0
			SST2-3B3/C	0	0	1	0
			SST2-3M1/C	12	0	0	0
			SST2-3M2/C	0	0	0	0
			SST2-3M3/C	0	0	1	0
			SST2-3E1/C	32	0	0	0
			SST2-3E2/C	5	0	0	0
			SST2-3E3/C	0	0	0	0
2010.8.15	Qindao	Effluent de-ballasting water of reference tank in cycle 3	SST3-3B1/C	3.5×10^5	2×10^2	19×10^2	9×10^2
			SST3-3M1/C	8.1×10^5	2×10^2	6×10^2	8×10^2
			SST3-3E1/C	2.4×10^6	90.00	14×10^2	4×10^2

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Appendix 4 **Results for microbes of shipboard trails of *BSKY*™**

Sampling date	Site of trials	Cycles of trials	Number	H.bacteria（CFU/100mL）	V.cholerae（CFU/100ml）	E.coli（CFU/100ml）	I.enterococci（CFU/100ml）
2010.1.21	Dongguan	Influent ballasting water of reference tank in cycle 4	SST1-4B1/C	1.3×10^5	6.9×10^2	3.7×10^3	10×10^2
			SST1-4M1/C	1.6×10^5	8.6×10^2	4.7×10^3	9×10^2
			SST1-4E1/C	1.1×10^5	7.6×10^2	3.3×10^3	13×10^2
2010.1.28	Qindao	Effluent de-ballasting water of treated tank in cycle 4	SST2-4B1/C	0	0	0	0
			SST2-4B2/C	0	0	0	0
			SST2-4B3/C	0	0	0	0
			SST2-4M1/C	0	0	0	0
			SST2-4M2/C	0	0	0	0
			SST2-4M3/C	0	0	0	0
			SST2-4E1/C	0	0	0	0
			SST2-4E2/C	0	0	0	0
			SST2-4E3/C	0	0	0	0
2010.1.28	Qindao	Effluent de-ballasting water of reference tank in cycle 4	SST3-4B1/C	2.1×10^5	1.7×10^3	3.2×10^4	8×10^2
			SST3-4M1/C	1.9×10^5	1.7×10^3	2.7×10^4	7×10^2
			SST3-4E1/C	2.0×10^5	1.7×10^3	2.9×10^4	11×10^2

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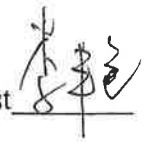
Appendix 5

Detect the photosynthesis efficiency of plankton in water samples of shipboard trials with a PAM analyzer （Fv/Fm）

Cycle 1 of the shipboard trials Qingdao-Dongguan(7.19-7.24）						
Ballast in Qingdao (7.19)					Average	SD
Influent water	SST3-1B1	0.58	0.57	0.56	0.57	0.009
	SST3-1M2	0.56	0.57	0.56		
	SST3-1E3	0.58	0.57	0.58		
De-ballast in Dongguan（7.24）						
Treated tank	SST2-1B1	0.00			0.01	0.005
	SST2-1B2	0.00				
	SST2-1B3	0.01				
	SST2-1M1	0.00				
	SST2-1M2	0.01				
	SST2-1M3	0.00				
	SST2-1E1	0.00				
	SST2-1E2	0.01				
	SST2-1E3	0.02				
Reference tank	SST3-1B1	0.21			0.19	0.02
	SST3-1M2	0.17				
	SST3-1E3	0.19				
Cycle 2 of the shipboard trials Dongguan-Qingdao（7.24-7.29）						
Ballast in Dongguan(7.24)						
Influent water	SST1-2B1	0.68			0.67	0.012
	SST1-2M2	0.66				
	SST1-2E3	0.68				
De-ballast in Qingdao（7.29）						
Treated tank	SST2-2B1	0.01			0.016	0.011
	SST2-2b2	0				
	SST2-2b3	0.01				
	SST2-2M1	0.02				
	SST2-2M2	0.03				
	SST2-2M3	0.01				
	SST2-2E1	0.02				
	SST2-2E2	0.03				
	SST2-2E3	0.02				
Reference tank	SST3-2B1	0.65			0.65	0.006
	SST3-2M2	0.66				
	SST3-2E3	0.65				

Analyst  Proofreader